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# ANATOMY AND SORUS DEVELOPMENT OF CAMPTOSORUS RHIZOPHYLLUS

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## Introduction

*Camptosorus rhizophyllus* (L.) Link is a native of eastern and central United States and Canada. There is one other species in the genus, *C. sibiricus* Rupr., a native of Siberia, China, and Japan. *C. rhizophyllus* grows in shady places, particularly on calcareous rocks, where the plants may form dense mats of overlapping fronds.

The general opinion that *C. rhizophyllus* is found chiefly on calcareous rocks led WHERRY (45, 46) to investigate whether the presence of this plant was diagnostic of such rock. In many regions he found the plant growing on rocks varying in lime content from 53.8% to less than 0.1%. The soils supporting its growth, however, are highly calcareous—having more lime than the average field soil—with reference to both total and soluble lime content of the soil. He concludes that this plant does not necessarily indicate the presence of calcareous rock but of calcareous soil.

*C. rhizophyllus* and *C. sibiricus* have had a varied history in classification, as is shown in CHRISTENSEN'S Index (8): *Asplenium* by LINNAEUS, 1753 (28); *Camptosorus rhizophyllus* by LINK, 1833; *C. sibiricus* by RUPRECHT, 1845; *Scolopendrium* by ENDLICHER, HOOKER and BAKER (25), CHRIST (7), and DIELS (18); *Antigramma* by J. SMITH; and *Phyllitis* by O. KUNTZE. It is now separated generically from *Phyllitis*, the genus which at present includes all species of *Antigramma* and *Scolopendrium*. The resemblance between *Camptosorus* and *Phyllitis* is indicated by their history in classification. Both ferns have geminate sori on the lateral veins, with indusia opening toward each other.

The groups which offer the closest similarity to *Camptosorus* are the Asplenioids and Blechnoids because of the linear, superficial sori. CHRISTENSEN (9), basing his conclusions largely on habit, places *Camptosorus* and *Phyllitis* in the Asplenioids. BOWER (3, 4, 5) allies them to the Blechnoideae. In the Asplenioids the sori are on lateral veins, but the indusia all open in the same direction, or, as in *Diplazium*, away from each other. In the Blechnoids the sorus is a coenosorus or chain of sori, with the indusia opening toward the midrib. BOWER traces the formation of the soral type of arrangement in *Camptosorus* and *Phyllitis* through the breakdown of the coenosorus, as seen in *Blechnum punctulatum* var. *krebsii*, in which the coenosorus has arched outward and been partially disrupted in those leaves changing from a narrow highly pinnate form toward a broad expanse. *Camptosorus* shows gradation from irregularly arranged sori at the base of the leaf to a sorus reminiscent of the Blechnoid coenosorus at the caudate tip of the leaf.

Certain aspects in the life history of *C. rhizophyllus* have been investigated by various workers: soral arrangement by the early taxonomists, by BOWER (3, 4, 5), and by CHRISTENSEN (9); vegetative reproduction by GOEBEL (20),

KUPPER (27), MCVEIGH (29, 30), and YARBROUGH (48); the possibility of hybridization between *C. rhizophyllus* and species of *Asplenium* by BRAUN (6), MAXON (31), HOYT (26), COULTER (12), DAVENPORT (13, 14), COPELAND (10), UNDERWOOD (44), SLOSSON (42), and BENEDICT (2); development of the prothalli by PICKETT (38) and WUIST (47); the resistance of the prothalli to desiccation by PICKETT (37, 39); and the type of stomata by COPELAND (11).

With the discussion of vegetative reproduction and of hybridization in *Camptosorus*, some mention of anatomical structure is found. YARBROUGH (48) describes briefly the rhizome apex and leaf primordia; MCVEIGH (30), the apical growth of leaves; and SLOSSON (42), the dermal appendages. HAYATA (22, 23) records observations on the anatomy of the stem of *C. sibiricus*. The stele is a radial dictyostele, with a single leaf trace bundle. A single root trace branches from the meristele just below the origin of the leaf trace strand.

The present investigation of the details of the anatomy of rhizome, leaf, and root, and of the early development of the sorus of *C. rhizophyllus* supplements the few anatomical facts already known about this species. Relationships between *Camptosorus* and other genera are elucidated when such anatomical details are compared.

## Material and methods

*Camptosorus rhizophyllus* was collected on the eastern slope of Mount Toby, Massachusetts, during 1939. Leaves were killed in Navashin's solution, rhizomes in a chromo-acetic solution containing 1% chromic acid and 3% acetic acid. Rhizomes were treated with 10% hydrofluoric acid before imbedding. All material was imbedded in paraffin and sectioned at 7–10 $\mu$. Stains used were Heidenhain's iron-alum haematoxylin counterstained with safranin, safranin-fast green, and safranin-gentian violet.

Diagrams of every tenth section of a cross-sectional series of the rhizome were made, and from these a clay model of the vascular system was built up.

## Observations

HABIT.—The stem of *C. rhizophyllus* is short, upright, and gives rise to the simple leaves in spiral succession. The leaves vary somewhat but in general are small and often gradually narrowed from a cordate or auricled base to a long slender acuminate tip which may root (fig. 1). Overall length of the blade may be 4–14 cm. The petiole, 1–6 cm. in length, is green, slightly winged for some distance below the blade, and bears dark brown acuminate scales at the base. The venation is partially reticulate, but toward the margin and the tip of the blade the veins are free.

The sori are oblong or linear and irregularly scattered on the reticulated veins; those next to the midrib are single, while the outer ones are inclined to approximate in pairs, so that the two indusia open face to face, or become confluent at their ends, forming a V-shaped sorus (fig. 1).

RHIZOME.—As determined from both sections and surface views, the growing tip of the rhizome is protected by many scales and by the surrounding leaf bases. The apex itself is flattened and has only a suggestion of conical configuration (fig. 3). An apical cell with three cutting faces is present (fig. 2). Leaf primordia are seen close to the apical cell and are formed from segments of it (figs. 2, 3).

In studying the cellular structure of the rhizome apex, occasionally one or two spiral tracheids may be seen ma-

turing just a short distance behind the growing region. In many cases, however, the first tracheids to mature are scalariform but without strong lignification. The scarcity of spiral or annular tracheids may be related to the slow growth of the rhizome. The protoxylem cells are few and scattered irregularly through the meristele. The metaxylem cells are scalariform, the first formed often being small in size. The first tissues of the stem to mature are the endodermis and phloem. The phloem cells are elongated, very small in cross section, and have slightly thickened walls. The endodermal cells are thin-walled, except for Caspary's strips on the radial walls.

The rhizome has an unperforated dictyostele, with curving meristeles bordering the leaf gaps (fig. 4). Cross sections of a mature rhizome show the number of meristeles to be ordinarily three, although two or four may be found (fig. 5*a–f*). There are two traces in each leaf base, arising on opposite sides of the leaf gap, which is formed by the division of a single meristele into two strands. Each strand may give rise to a leaf trace bundle immediately (fig. 5*e* top), while uniting with an adjacent meristele (fig. 5*e* bottom), or after uniting with an adjacent meristele. In general, one meristele gives off a leaf trace bundle before merging, while the other gives off one while merging—or after it has merged with an adjacent strand. The leaf trace bundles diverge from the meristeles on either side of the leaf gap just above its opening, but they do not always arise at the same level. The root trace associated with the leaf base usually arises from the meristele as it divides to form the leaf gap (fig. 4); occasionally it may arise from one of the meristeles shortly after the leaf gap is formed (fig. 4, *rt tr* 1).

Each meristele (fig. 6) is delimited from the cortex by a continuous endodermis. A layer of pericycle, one to two cells thick, lies between the endodermis and the phloem. The xylem, more or less

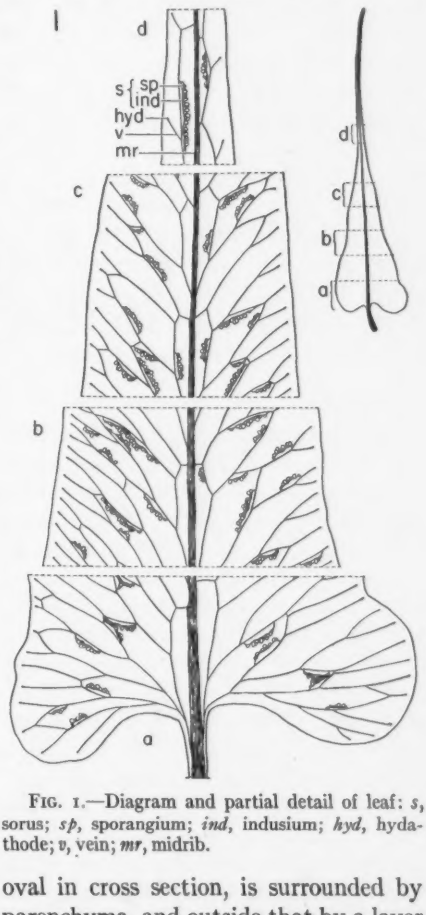
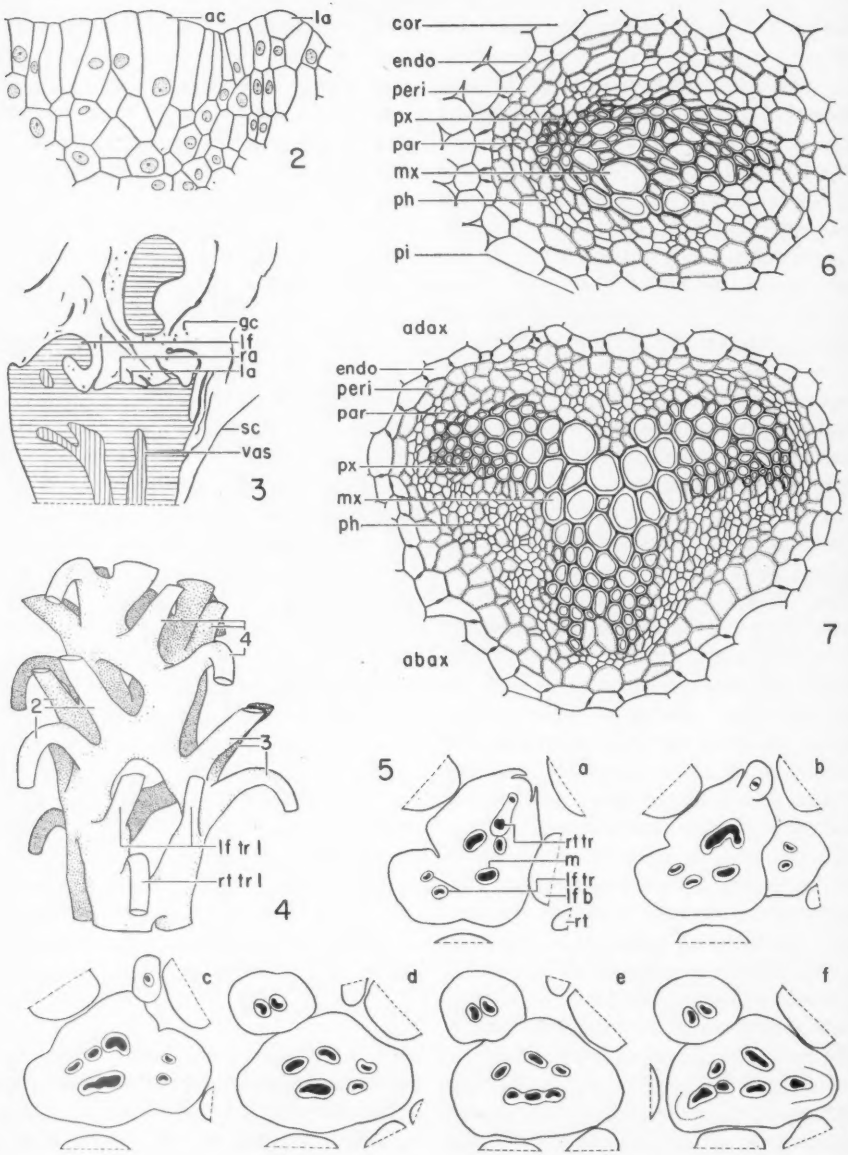



FIG. 1.—Diagram and partial detail of leaf: *s*, sorus; *sp*, sporangium; *ind*, indusium; *hyd*, hydathode; *v*, vein; *mr*, midrib.

oval in cross section, is surrounded by parenchyma, and outside that by a layer of phloem which is not always continuous. The protoxylem cells are few and scattered and not noticeable in a mature meristele. Small metaxylem cells are found at the two ends of the oval core and to some extent along the cortical side. The larger metaxylem cells are toward the pith.




FIGS. 2–7.—Fig. 2, apex of rhizome: *ac*, apical cell; *la*, leaf apex. Fig. 3, diagram of longisection of rhizome through apex: *gc*, glandular cell; *lf*, young leaf; *ra*, rhizome apex; *sc*, scale; *vas*, vascular system. Fig. 4, reconstructed vascular system; *lf tr*, leaf trace; *rt tr*, root trace; in order of formation: 1, first; 2, fourth; 3, third; 4, sixth; opening of nine leaf gaps included in model. Fig. 5, cross sections of rhizome (*a–f*): *m*, meristele; *lf b*, leaf base; *rt*, root. Fig. 6, detail of meristele: *cor*, cortex; *endo*, endodermis; *peri*, pericycle; *px*, protoxylem; *par*, parenchyma; *mx*, metaxylem; *ph*, phloem; *pi*, pith. Fig. 7, detail of vascular bundle of upper petiole and midrib of leaf: *adax*, adaxial side of bundle; *abax*, abaxial side.

In cross section each vascular strand of the leaf trace is crescent-shaped, the concave side facing diagonally outward. Spiral tracheids, making up the protoxylem of the traces, are located at the tips of the crescent, but those first formed are one or two cells back from the tip on the convex surface. The larger metaxylem cells are found in the center of the convex curve, on the adaxial side of the bundle. In the rhizome and part way through the petiole the two strands remain distinct (fig. 8). As they approach the leaf blade, first the endodermis becomes continuous around the two bundles (fig. 9), then the phloem does not develop between the xylem tracts, and finally the latter become contiguous (fig. 10), in which condition the xylem is found in the midrib of the leaf. The merging reduces the number of protoxylem points to three as the two abaxial groups and part of the adjacent metaxylem approach each other and become indistinguishable, forming a trace V-shaped in cross section. The two strands do not completely lose their identity. Occasionally the adaxial tips are not completely joined, showing a slight tendency to form an X-shaped trace (fig. 7). Phloem is found in greatest amount in the concavities of the trace where it matures first, but it is also present around the whole trace in form of a few scattered cells.

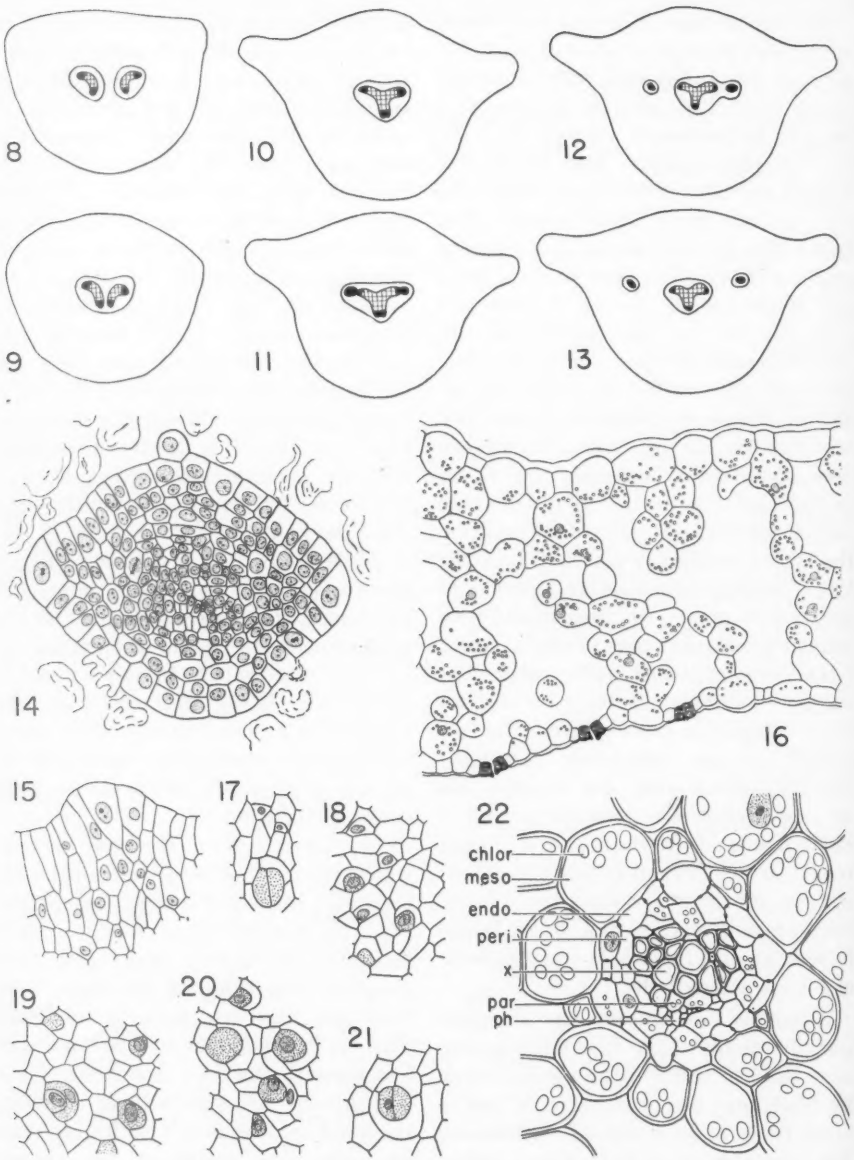
Although *Camptosorus* has a simple leaf, the origin of the first veins arising from the leaf trace in the petiole below the blade may be compared with that of pinna-traces of ferns with pinnate leaves. The adaxial corners of the xylem strand gradually diverge from the main strand, but the other tissues remain continuous for a longer time (figs. 11, 12). Each of the newly diverged lateral veins (fig. 13) is made up of a more or less flattened strand of xylem, with phloem largely on the abaxial side but with a few scattered cells on all sides. It is surrounded by a complete endodermis and pericycle.

LEAF.—The leaf arises about seven cells away from the apical cell of the rhizome. The first evidence of a leaf primordium is an upward bulge at one side of the stem apex, at the tip of which is a distinct wedge-shaped cell (figs. 3, 15). As the leaf develops a series of meristematic cells extends from the leaf apex back in two lateral rows along the leaf margin. The broadening of the leaf is brought about by the marginal series of cells cutting off segments alternately from the upper and lower side (fig. 14). The young leaf gives rise to multicellular glandular hairs (fig. 14).

While the petioles of *Camptosorus* are green and small air spaces are common in the cortex, there are no stomata in the epidermis except on the abaxial surface of the lateral wings. In the wings the intercellular spaces are larger and communicate directly with those of the cortex of the petiole proper. The stomata on the abaxial faces open into the intercellular spaces of the wing.

The mesophyll of the mature leaf, which regularly is seven cells thick, is composed entirely of spongy parenchyma (fig. 16). The spongy cells in the lower half may be slightly larger and more irregular than those in the upper half; they may also have fewer chloroplasts. The upper epidermal cells are more or less regular, contain a few chloroplasts, and are covered with a layer of cutin; the lower epidermal cells, which are more irregular, also contain a few chloroplasts but have less cutin.

Stomata are found only in the lower epidermis, the long dimension of each parallel to the length of the leaf. The guard cells have many chloroplasts. Each



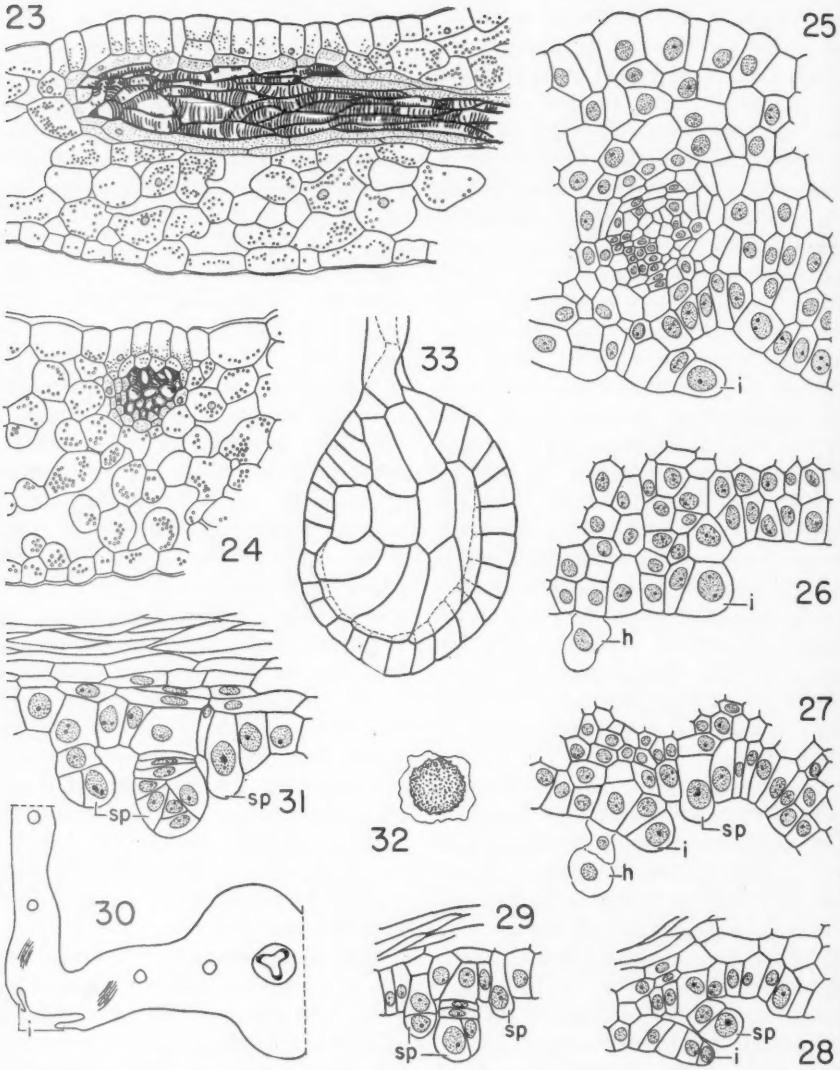

FIGS. 8–22.—Figs. 8–13, cross sections of petiole from base to leaf showing origin of first lateral veins. Fig. 14, cross section of young leaf showing marginal meristematic cells and many hairs. Fig. 15, detail of longisection of leaf primordium showing apical cell. Fig. 16, cross section of mature leaf near midrib showing spongy character of mesophyll and stomata in lower epidermis. Figs. 17–21, surface views of lower epidermis of young leaves showing development of guard cells. Fig. 22, detail of lateral vein in leaf: *chlor*, chloroplast; *meso*, mesophyll cell; *x*, xylem.

pair of guard cells is almost surrounded, except for one end, by a single epidermal cell. Development of the stomatal apparatus is similar to that described and illustrated by ATKINSON (1) and SADEBECK (40) for many ferns. In an epidermal cell of a young leaf a corner is cut off; this is followed by differential growth of the two cells, the outer growing faster than the guard cell initial. The latter grows more on the side toward the epidermal cell, so that the initial becomes surrounded. This initial divides in half, parallel to the long axis of the leaf, forming the guard cells (figs. 17–21). A cross section of a pair of such cells shows moderate guard cell thickening (fig. 16). The cutin on the lower epidermis is not so heavy as on the upper but is heavier on the guard cells, where it forms a little ridge seen in cross section on the outer face of the cells around the stoma. Around the margin of the leaf the cell walls of epidermal and mesophyll cells thicken; there is less intercellular space, and the cutin becomes much heavier.

The structure of the midrib is like that of the petiole which has been described. The small lateral veins diverging from the midrib are almost terete and are surrounded by a complete endodermis (fig. 22). A pericycle, not always continuous, is present. The xylem is largely spirally thickened, but in the larger veins tracheids with scalariform thickenings are found. A small amount of phloem is present on the abaxial side of the bundle. The lateral veins are located near the middle of the tissues of the leaf, but as the vein approaches the leaf margin it rises to the surface and the number of tracheids increases (figs. 23, 24). Cells of the upper epidermis immediately above this enlarged tip of the vein are thin-walled and not covered with cutin, as is the rest of the leaf surface. This simple type of hydathode is formed on the end of every lateral vein, even in the acuminate tip of the leaf. The hydathodes are oval in outline, and the length varies from one and a half to three times the width. As seen in section (figs. 23, 24), the margins of the hydathodes may be slightly depressed, but the central portion is as high as or higher than the surrounding leaf surface. Attempts to induce guttation were not successful.

SORUS.—The sori are irregularly placed on the reticulated veins. Occasionally there are sori back to back on one vein, but in general, if they are paired at all, they are seated on separate veins and face one another (fig. 1). On the lateral veins, if they occur singly, they may either face toward or away from the midrib. They are often found on the veins running parallel to the midrib, with the indusium opening toward the midrib, particularly near the tip of the leaf, where they may reach a length of 5 mm. (fig. 1*d*). When young, the sorus is covered by a membranous indusium which opens along the side opposite the vein on which the sorus is seated. As the sorus matures the indusium pulls back, and the long-stalked sporangia protrude from beneath it.

The sorus makes its appearance on the under surface while the leaf is still very small but after the vascular tissue of the main vein has begun to mature and the lateral veins to differentiate. The first change indicating development of the sorus is evident directly below a vein, where the indusium forms a ridge along the margin of a slight depression on the lower surface of the leaf (fig. 26). The cells of the ridge divide, producing a definite flap (figs. 25, 27, 28, 30). The indusium remains one cell in thickness




FIGS. 23–33.—Fig. 23, longisection of hydathode. Fig. 24, cross section of hydathode. Figs. 25–29, 31, development of sorus: *i*, developing indusium; *sp*, developing sporangium; *h*, hair. Fig. 30, cross section of young leaf showing two developing sori facing each other. Fig. 32, mature spore. Fig. 33, full-sized but still immature sporangium.

through most of its expanse but may become two or three cells thick at the base. Young indusial flaps, opening toward one another and each seated on a separate vein, are frequently seen (fig. 30).

While the indusium is still very small certain epidermal cells at its base become conspicuous owing to the enlarged nuclei (fig. 25). At first there is a suggestion of basipetal sequence in the appearance of these sporangial initials, but later they are definitely mixed. Instead of forming on a well-developed receptacle, the initials arise from cells directly under a vein which is near the lower surface of the leaf in the region of the sorus (figs. 25, 29, 30).

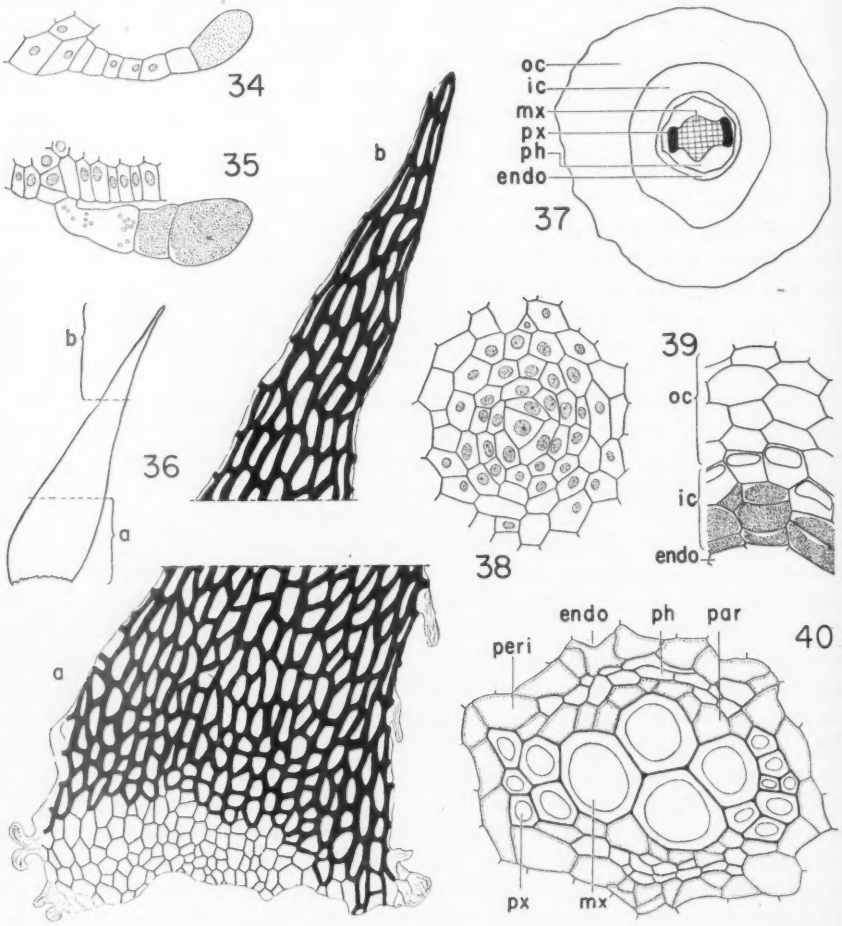
The epidermal cell from which the sporangium will arise possesses a large nucleus, stains more readily, and soon becomes papillate (figs. 27, 29, 31). The first division of this projecting cell is parallel to the surface of the leaf, forming a stalk cell which protrudes slightly beyond the epidermis, and a terminal cell (fig. 29). The stalk cell may undergo further divisions parallel to the leaf surface, but the cells thus formed do not lengthen until later (figs. 28, 29, 31). Then by successive oblique divisions in the end cell a tetrahedral cell is formed at the tip (figs. 29, 31). The outer portion of this is cut off, leaving an inner sporogenous initial surrounded by a layer of sterile cells (fig. 31). Subsequent development follows the usual pattern, and the mature sporangium has an annulus that is vertical and interrupted (fig. 33). The cell next to the stalk is not indurated. The number of indurated cells is variable; in different sporangia sixteen to twenty-seven were counted, with an average of twenty in twenty-three sporangia. There are four stomium cells which are broadened laterally. In the development of the young sporangium the series of cross walls in the stalk cell produces a one-rowed stalk rather than the three-rowed type which ordinarily develops by a series of oblique divisions. In the mature sporangium, the greater length of the stalk consists of a single row of cells which passes over into two or three where it joins the capsule. The mature spores are bilateral, with a dark brown, slightly rough coat and a perispore (fig. 32).

Dermal appendages.—Both hairs and scales are found on *C. rhizophyllus*. Multicellular hairs with a large glandular cell at the tip are common on both surfaces of the young leaves (fig. 35) but disappear from the upper surface as the leaf matures, although a few may persist on the lower surface, where they appear as dark brown spots. The rhizome tip and petiole bases are covered by glandular hairs (fig. 34) and by scales about 1 mm. in length and 0.3 mm. in width at the base. The scales (fig. 36) are narrow and acuminate, with thin outer walls and with the lateral walls heavily thickened. They are one cell in thickness from base to tip and are attached by several cells at the base. In most cases the tip ends in a large glandular cell similar to that of the hairs on the leaf.

Root.—A single root is associated with a leaf base. It diverges endogenously from a meristele, usually below—but occasionally above—the opening of the leaf gap (fig. 4). The root primordium can be seen arising close to the primordium of the leaf with which it is to be associated. Protoxylem, chiefly small scalariform tracheids, does not appear until the root has progressed far enough through the cortex to break the epidermis. At the tip of the root is a single pyramidal apical cell (fig. 38). Divisions of this cell in three planes give rise to cells the derivatives of which form the

vascular, cortical and epidermal tissues of the root, while divisions on the fourth side give rise to the root cap.

pass outward, either directly or obliquely. In a few instances roots have been observed to branch immediately after they




FIGS. 34–40.—Fig. 34, glandular hair from rhizome and petiole base. Fig. 35, glandular hair from leaf. Fig. 36, diagram and partial detail of scale from rhizome and petiole base; glandular tip not shown. Fig. 37, cross section of root: *oc*, outer cortex; *ic*, inner cortex. Fig. 38, detail of cross section of root apex showing apical cell. Fig. 39, detail of cortex of root. Fig. 40, detail of stele of root.

The root may remain within the leaf base with which it is associated for a considerable distance before it becomes free and turns downward, or it may bend downward in the cortex almost immediately after it is given off and then become free from the rhizome. The roots may be 0.5 mm. in diameter but are often less.

Several layers of cortical cells of the root near the endodermis are more or less thickened on their radial and inner

tangential walls (figs. 37, 39). A continuous endodermis separates the stele from the cortex (figs. 37, 40). The pericycle is continuous and usually only one row of cells in thickness. The xylem is diarch; the two protoxylem strands are in contact with the pericycle. A small amount of phloem is present on either side of the xylem and separated from it by a few parenchyma cells.

### Discussion

The two species of *Camptosorus*, when not looked upon as forming a distinct genus, have often been placed in one of the genera now included in the genus *Phyllitis*. *P. scolopendrium*, the best known species of the genus, is larger than *Camptosorus* and has simple leaves growing in spiral succession from an upright stock. The scales found on the rhizome tip and leaf bases have a glandular cell at the tip (3), as do those found in *C. rhizophyllus*. In some species of *Phyllitis*, as *P. scolopendrium*, the venation is free, but in others, such as the Mexican species *P. nigripes* (Fée) O. Ktze, the venation is reticulate (9, 7). The rhizome of *P. scolopendrium* contains a radial dictyostele with a single root trace given off below the opening of a leaf gap. The leaf gap opens above the origin of the root trace and from each margin a strap-shaped vascular strand is given off (3, 22, 23). In the leaf stalk the strands may fuse, forming an X-shaped trace in which the upper arms of the figure are stronger (34, 35, 36, 43). The origin of the lateral veins is from the adaxial tips of the trace. The petiole of *P. scolopendrium* has small lateral wings for a short distance below the blade and stomatal strips for a longer distance (32). The leaf blade is similar to that of *C. rhizophyllus* in having chloroplasts in the epidermal cells and no palisade tissue; the mesophyll is nine cells thick (19, 43, 34, 35). A close resemblance to *C. rhizophyllus* is found in the soral arrangement and development and in the sporangium and spores of *P. scolopendrium*. The soral arrangement of *C. rhizophyllus* is less regular than that of *P. scolopendrium* but is strikingly like that of *P. nigripes*, which has reticulated veins (7, fig. 672). BOWER (3, 4) describes the origin of the sorus on the young leaf of *Phyllitis:* the indusium arises at the edge of a slight depression, and the sporangia arise at the base of the depression in a sequence at first slightly basipetal (3). The mature sporangium of *Phyllitis*, described by STRASBURGER (43), has an incomplete vertical annulus with a varying number of indurated cells and a stalk which passes over from a single to a double row of cells. The spore is bilateral and has a rough coat and perispore (21).

The resemblance between *Camptosorus* and *Phyllitis* consists in the habit of the two ferns, in the scales with a glandular tip, in the unperforated dictyostele with binary leaf traces and single root associated with a leaf base, in the geminate arrangement of sori, in the development of the sorus at first basipetal and then mixed, in the early appearance of the indusium, in the sporangium with stalk one-rowed at the base, and in the bilateral spore with perispore. As the relationship between *Camptosorus* and *Phyllitis* seems highly probable, the two genera will be discussed together.

The two groups to which they would naturally be compared are the Asplenioids and the Blechnoids because of the elongated, superficial sori. The relationship of these groups to each other is not established. BOWER (4), who classes *Camptosorus* and *Phyllitis* with the Blechnoids, considers that similarities

between this group and the Asplenioids afford a good example of convergent evolution. On the other hand, CHRISTENSEN (9) places the two genera in the Asplenioids, which group he places between the Dryopteroids and the Blechnoids.

In habit the Blechnoids vary from small forms to some that are subarborescent. The smaller forms, which may have simply pinnatifid leaves, show similarity to *Camptosorus* and *Phyllitis* in habit and anatomy. The dermal appendages are broad scales and hairs with a large terminal glandular cell. The vascular system is dictyostelic, the number of overlapping leaf gaps depending on the size of the fern. In *B. patersoni*, one of the smaller forms, a single root trace passes off just below the base of the leaf gap, and above this from right and left of the gap two equal leaf traces arise (3); this is similar to that described for *C. rhizophyllus*. DAVIE (15–17) has found that many species of *Blechnum*, including *B. patersoni* and *B. punctulatum* var. *krebsii*, have an "extra-marginal" type of pinna-trace. In this type of trace the portion which comes from the adaxial side of the leaf trace is nipped off from the back of the hook, the adaxial face of the curved leaf trace; the extreme tip of the adaxial portion is continued upward as part of the leaf trace. This differs from *Camptosorus* and *Phyllitis*, which give rise to the first lateral veins in a "marginal" manner.

BOWER bases his classification of *Camptosorus* and *Phyllitis* among the Blechnoids on the interpretation of the phyletic development of the sorus, with emphasis on the intermediate conditions between the Blechnoid type of sorus and that of *Camptosorus* and *Phyllitis* found in *B. punctulatum* var. *krebsii*. HOOKER (24) was the first to suggest the similarity of this species of *Blechnum* to *Phyllitis*. *Camptosorus* offers the most interesting comparison, as its sori vary from parallel ones of the Blechnoid type in the excurrent apex of the leaf to soral arches in the middle region and irregular arrangement (single, paired face to face, paired back to back) in the basal portions —conditions very nearly matched in *B. punctulatum* var. *krebsii* (4). The sorus of *Blechnum* is like that of *C. rhizophyllus* in showing a slightly gradate sequence at first but differs from it in the formation of the indusium after the sporangia have appeared. The sporangia of the species of *Blechnum* studied by SCHNARF (41) differ from those of *C. rhizophyllus* in having a three-rowed stalk, but the spores are bilateral and have a perispore (21) as in the latter species.

The genus of the Asplenioids which *C. rhizophyllus* most closely resembles in habit is *Asplenium*. Many of the latter genus are rock-loving ferns with simple to decompound leaves. They often propagate vegetatively, as does *Camptosorus*. Mature guard cells in the leaves of *Camptosorus* and species of *Asplenium* are alike (11). The lattice-thickening of the scales on the rhizome of species of *Asplenium* is like that of the scales of *C. rhizophyllus*. The rhizomes of such species as *A. alatum*, *A. obtusatum*, and *A. marginatum* show construction similar to that of the walking fern. The axis contains a dictyostele; the two straps of the leaf trace arise from either side of the leaf gap and the root traces from the meristele below the opening of the leaf gap. The leaf trace bundles may fuse upward to form a vascular tract, X-shaped in cross section (3, 4, 34, 35). DAVIE (15–17) has found that species of *Asplenium* (*A. bulbiferum*, *A. obtusatum*, *A. ruta-muraria*, *A. trichomanes*, *A. pinnatifidum*) have a "marginal" type of

pinna-trace. In the marginal type the first indication of the preparation for departure of the pinna-trace, as the leaf trace is followed up the petiole, is an extension of the mass of tracheids on the adaxial face of the petiole, that portion of the trace nearest the pinna. This tip is cut off to supply the pinna. This is similar to *Camptosorus* and *Phyllitis*, which, although not pinnate, give off the first lateral veins in a marginal manner.

Typically the sorus of *Asplenium* arises on the fertile vein, and the indusium opens so that it faces the midrib of the fertile frond, pinna, or pinnule. Development of the sorus is like that of *C. rhizophyllus* in the appearance of the indusium before the sporangia, but differs in that the sporangia arise in a distinctly mixed sequence, with no suggestion of basipetal arrangement (3). Sporangia, illustrated by SCHNARF (41) and MÜLLER (33), have a long, one-rowed stalk similar to that found in *C. rhizophyllus*. The spores are bilateral, with a perispore (21).

*C. rhizophyllus* and *Phyllitis* show close resemblance to both *Asplenium* and small forms of *Blechnum* in habit, in the presence of scales on the rhizome, in the unperforated dictyostele with binary leaf traces, in the single root associated with a leaf base, and in the bilateral spore with perispore. The arrangement of the sori on the mature leaf of *C. rhizophyllus* and of *Phyllitis* is like that of *B. punctulatum* var. *krebsii*. A glandular cell is found at the tip of the scales in *C. rhizophyllus*, in *Phyllitis*, and in *Blechnum*. Similarity between *C. rhizophyllus* and *Asplenium* is found in the lattice-thickened scales, in the crescent-shaped leaf trace bundles fusing in the petiole or midrib of the leaf, in the marginal type of pinna-trace, in vegetative reproduction, and in the sporangium with a one-rowed stalk. Relationship is also suggested by the possibility of hybridization between the two genera (2, 6, 10, 12, 13, 14, 26, 31, 42, 44). Although there is more evidence for allying *Camptosorus* with the Asplenioids than with the Blechnoids, difficulty is found in the interpretation of the phyletic development of the soral arrangement. There is no transition form between *Asplenium* and *Camptosorus*, such as *Blechnum punctulatum* var. *krebsii* provides between *Blechnum* and *Camptosorus*. However, more will need to be known about both species of *Camptosorus*, about *Phyllitis* (particularly those species with reticulate venation), and about various species of both *Asplenium* and *Blechnum* before the relation of the Asplenioids and Blechnoids to each other and the systematic position of *Camptosorus* and *Phyllitis* can be determined with any degree of certainty.

## Summary

1. The rhizome of *Camptosorus rhizophyllus* is radial, with leaf bases diverging from all sides. It has an unperforated dictyostele with curving meristeles bordering the leaf gaps. In transverse section ordinarily three meristeles are found.

2. The two parts of the binary leaf trace arise individually in the lower half of the leaf gap, one from each side of it. They merge in the petiole, forming a trace V-shaped in cross section, or occasionally with a suggestion of an X-shape.

3. The lateral veins arise from the margin of the adaxial arms of the leaf trace or midrib, a marginal type of pinna-trace.

4. A single root is associated with a leaf base. The root trace diverges from a meristele just before it divides to form

the leaf gap of the leaf with which the root is associated.

5. Dermal appendages are of two types—multicellular glandular hairs on young leaves, with a few persisting on the lower surface of mature leaves; and lattice-thickened scales, often with a glandular tip, on the petiole base and the rhizome tip.

6. Leaves are covered with a heavy coat of cutin; the epidermal cells contain chloroplasts; and stomata are present in the lower epidermis; the mesophyll is not differentiated into spongy and palisade tissue. Simple hydathodes are found on the upper surface of the leaf near the margin at the tips of the lateral veins.

7. The sori are oblong to linear and are scattered irregularly on the reticulated veins, some borne singly and facing in different directions and some in pairs facing each other.

8. Each sorus is seated on a vein and covered by a membranous indusium opening along the side of the sorus away from the vein on which it is seated.

9. The sorus develops when the leaf is still young but after the midrib has begun to mature and the lateral veins to differentiate. The indusium appears first and is followed by development of the sporangia.

10. The sporangia appear in a sequence at first slightly gradate but later distinctly mixed.

11. The sporangia are variable in size, and the annulus contains a variable number of indurated cells, with an average of twenty. The stalk is one-rowed, passing over to two or three rows at the base of the capsule.

12. *Camptosorus* shows resemblance to both *Asplenium* and small forms of *Blechnum* in habit, dermal appendages, and rhizome anatomy; to *Asplenium* in leaf trace, pinna-trace, vegetative propagation, sporangia, and spores; and to *Blechnum punctulatum* var. *krebsii* in soral arrangement. There appears to be more evidence for allying *Camptosorus* (and what seems to be its closest relative, *Phyllitis*) to the Asplenioids than to the Blechnoids.

The writer expresses her thanks for the facilities of the plant science laboratories at Mount Holyoke College and for the helpful direction and criticism given by DR. ALMA G. STOKEY.

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## LITERATURE CITED

1. ATKINSON, G. F., The biology of ferns. New York. 1894.
2. BENEDICT, R. C., Do ferns hybridize? Science n.s. **33**:254–55. 1911.
3. BOWER, F. O., Studies in the phylogeny of the Filicales. IV. *Blechnum* and allied genera. Ann. Bot. **28**:363–432. 1914.
4. ———, The ferns. Vol. III. Leptosporangiate ferns. Cambridge. 1928.
5. ———, Primitive land plants. London. 1935.
6. BRAUN, E. LUCY, A new fern hybrid—*Asplenium cryptolepis* × *Camptosorus rhizophyllus*. Amer. Fern Jour. **29**:133–135. 1939.
7. CHRIST, H., Die Farnkräuter der Erde. Jena. 1897.
8. CHRISTENSEN, CARL, Index Filicum. Hafniae. 1906.
9. ———, Chap. XX: Filicinae (pp. 522–550). Manual of pteridology. Ed. by FRANZ VERDOORN. The Hague. 1938.
10. COPELAND, E. B., Two fern monstrosities. BOT. GAZ. **34**:142–144. 1902.
11. ———, The mechanism of stomata. Ann. Bot. **16**:327–364. 1902.
12. COULTER, J. M., and COULTER, M. S., Editorial. BOT. GAZ. **7**:37. 1882.
13. DAVENPORT, G. E., § 212. *Asplenium ebenoides* R. R. Scott. Bull. Torr. Bot. Club **6**:200. 1878.
14. ———, Two new ferns from New England. With some observations on hybridity and nomenclature. BOT. GAZ. **19**:492–497. 1894.
15. DAVIE, R. C., The pinna-trace in the ferns. Trans. Roy. Soc. Edinburgh **50**:349–387. 1914.
16. ———, On the leaf-trace in some pinnate leaves.

Trans. Roy. Soc. Edinburgh **52**:1–36. 1917–1918.

17. ———, A comparative list of fern pinna-traces, with some notes on the leaf-trace in the ferns. Ann. Bot. **32**:233–245. 1918.
18. DIELS, L., Polypodiaceae. In ENGLER and PRANTL's Die natürlichen Pflanzenfamilien. Leipzig. 1902.
19. GLOSS, MARY ELGIN, Mesophyl of ferns. Bull. Torr. Bot. Club **24**:432–435. 1897.
20. GOEBEL, K., Ueber Regeneration im Pflanzenreich. Biol. Central. **22**:385–397. 1902.
21. HANNIG, E., Über das Vorkommen von Perisporien bei den Filicinen nebst Bemerkungen über die systematische Bedeutung derselben. Flora **103**:321–346. 1911.
22. HAYATA, BUNZO, On the systematic importance of the stelar system in the Filicales. I. Bot. Mag. Tokyo **41**:697–718. 1927. (In Japanese.)
23. ———, Über die systematische Bedeutung des des stelären Systemes in den Polypodiaceen. Flora **124**:38–62. 1929–1930.
24. HOOKER, W. J., Species Filicum. Vol. III. London. 1860.
25. HOOKER, W. J., and BAKER, J. G., Synopsis Filicum; or a synopsis of all known ferns, including the Osmundaceae, Schizaeaceae, Marattiaceae, and Ophioglossaceae. 2d ed. London. 1874.
26. HOYT, W. D., Physiological aspects of fertilization and hybridization in ferns. BOT. GAZ. **49**: 340–370. 1910.
27. KUPPER, WALTER, Über Knospenbildung an Farnblättern. Flora **96**:337–408. 1906.
28. LINNAEUS, C., Species Plantarum. Vol. II. Holmiae. 1753. Facsimile edition. Berlin. 1907.
29. MCVEIGH, ILDA, Vegetative reproduction in *Camptosorus rhizophyllus*. BOT. GAZ. **95**:503–510. 1934.
30. ———, Apical growth of the leaves of *Camptosorus rhizophyllus*. Amer. Jour. Bot. **23**:669–673. 1936.
31. MAXON, W. R., Notes on the validity of *Asplenium ebenoides* as a species. BOT. GAZ. **30**: 410–415. 1900.
32. MITCHELL, MARGARET R., Note on the lateral lines of the petioles of ferns. Trans. Proc. Bot. Soc. Edinburgh **29**:105–112. 1924.
33. MÜLLER, CARL, Zur Kenntniss der Entwicklungsgeschichte des Polypodiaceensporangiums. Ber. deutsch. Bot. Ges. **11**:54–72. 1893.
34. PARMENTIER, M. P., Recherches sur la structure de la feuille des fougères et sur leur classification. Ann. Sci. Nat. Bot. 8 sér. **9**:289–361. 1899.
35. PELOURDE, FERNAND, Recherches anatomiques sur la classification des fougères de France. Ann. Sci. Nat. Bot. 9 sér. **4**:281–372. 1906.
36. ———, Recherches comparatives sur la structure des fougères fossiles et vivantes. Ann. Sci. Nat. Bot. 9 sér. **10**:115–147. 1909.
37. PICKETT, F. L., Resistance of the prothallia of *Camptosorus rhizophyllus* to desiccation. Bull. Torr. Bot. Club **40**:641–645. 1913.
38. ———, The development of the prothallium of *Camptosorus rhizophyllus*. BOT. GAZ. **57**:228–238. 1914.
39. ———, Some ecological adaptations of certain fern prothallia—*Camptosorus rhizophyllus* Link, *Asplenium platyneuron* Oakes. Amer. Jour. Bot. **1**:477–498. 1914.
40. SADEBECK, R., Pteridophyta. Vol. I, ENGLER and PRANTL, Die natürlichen Pflanzenfamilien. Leipzig. 1902.
41. SCHNARF, KARL, Beiträge zur Kenntnis des Sporangienwandbaues der Polypodiaceae und der Cyatheaceae und seiner systematischen Bedeutung. Sitz. Kais. Akad. wiss. mathnaturw. Klasse. **113–114**:549–573. 1904–1905.
42. SLOSSON, MARGARET, The origin of *Asplenium ebenoides*. Bull. Torr. Bot. Club **29**:487–495. 1902.
43. STRASBURGER, E., Handbook of practical botany. Ed. from German by W. HILLHOUSE. London. 1887.
44. UNDERWOOD, L. M., The habitats of the rarer ferns of Alabama. BOT. GAZ. **22**:407–412. 1896.
45. WHERRY, E. T., A chemical study of the habitat of the walking fern, *Camptosorus rhizophyllus* (L.) Link. Jour. Wash. Acad. Sci. **6**:672–679. 1916.
46. ———, Observations on the habitat of certain ferns. Amer. Fern Jour. **7**:110–112. 1917.
47. WUIST, ELIZABETH D., Branched prothallia in the Polypodiaceae. Bull. Torr. Bot. Club **43**: 365–383. 1916.
48. YARBROUGH, J. A., The foliar embryos of *Camptosorus rhizophyllus*. Amer. Jour. Bot. **23**:176–181. 1936.

# SPORULATION IN SACCHAROMYCES CEREVISIAE[1]

CARL C. LINDEGREN AND GERTRUDE LINDEGREN

## Introduction

Sporulation in yeasts was probably first studied by DE SEYNES (**19**), who reported that round spores found "in the surface of the water" germinated to produce elongate cells in a mixture of wine and water, but that these elongate cells produced spores again when transferred to a more dilute medium. REES (**16**) found that when yeast was planted on the cut surface of various vegetables (cooked or raw), growth continued until the fourth day, when budding stopped. On the fifth day the vacuoles in the cell disappeared and the protoplasm became coarsely granular. Spore formation occurred regularly on the sixth day. Spores also appeared on the sixth day if fresh yeast from beer vats or wine must was transferred to the cut surface of carrot or potato. REES did not find spores in old lagering vats or in compressed yeast, but washed yeast from these sources placed in a beaker in a layer approximately 4 mm. thick and protected from dust sporulated abundantly in about 3 weeks.

ENGEL (**4**) devised the classic method of inducing yeasts to sporulate by transferring the cells from a nutrient broth to plaster of Paris blocks. HANSEN (**7**) used this method successfully. GRAHAM and HASTINGS (**6**) have improved the technique considerably by making the gypsum slants in test tubes, thus reducing the danger of contamination. BEYERINCK (**2**, **3**) obtained yeast spores by transferring the suspensions to slants made from agar that had been fermented and subsequently washed. He preferred this method to use of the gypsum block, probably because less contamination was encountered.

HANSEN and BEYERINCK considered it essential that the yeast cells be well nourished before transferring them to the aerating surface, but they did not specify any nutrients aside from beer wort or grape must. They believed that sporulation occurred when the "well-nourished" cells were deprived of all nutrients, and HANSEN'S blocks and BEYERINCK'S purified agar were flooded with distilled water. They agreed that starvation conditions and an abundance of oxygen were essential.

GORODKOWA (**5**) developed an agar containing 1% peptone and 0.25% glucose. On this medium sporulation occurred after 3–4 days. She showed that on a similar medium containing 5% glucose no spores were formed, indicating that the "starvation" conditions essential to sporulation involved primarily the sugars in the substrate rather than other components.

WELTEN (**20**) challenged the view that starvation was essential to sporulation. He found that on prune extract agar, yeasts sporulated well. He even doubted the necessity for oxygen, since in his experiments, colonies imbedded in the agar sporulated as well as those on the surface. He found that yeasts grown in pear extract or beer wort did not sporulate so well as those grown on prune extract agar. But sporulation occurred when the washed yeast grown on prune extract was placed on glass plates, filter paper, or sterile washed agar slopes, if a drop of prune extract were added. If no prune extract were added, no spores were produced. GORODKOWA'S and WELTEN'S work proved that starvation alone is not the complete explanation of the phenomenon. WELTEN showed further that acidity of the medium in which sporulation occurred was important, no

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spores occurring in an alkaline milieu. WELTEN also found that more spores were produced in concentrated than in dilute prune extract, also that a small amount of $MgSO_4$ aided sporulation. Young cells were not essential; those 3–4 days old sporulated better than those 1–2 days old.

BALTATU (1) found that spores were produced by mycodermas in grape juice but usually germinated immediately, so that they could be detected only by periodic examination of the culture. The addition of very small amounts of acetic acid and grape juice to the water in which the gypsum blocks were placed increased sporulation.

MRAK (13) discovered that many yeasts sporulated well on the slopes of an agar medium containing a mixture of vegetable (cucumber, beet, potato, and carrot) extracts. This is clearly a medium containing only a small amount of sugar, which is probably soon exhausted to about the concentration of GORODKOWA'S.

NICKERSON and THIMANN (14) found that conjugation and sporulation occurred more abundantly in a *Zygosaccharomyces* when many dead cells were present and postulated some stimulating substance derived from the dead cells. MANEVAL (12) found that sporulation occurred in *Saccharomyces* on the outside of compressed yeast cakes, but he did not comment on the presence of dead cells. NICKERSON and THIMANN also discovered that an extract from *Aspergillus* increased conjugation and sporulation, and later (15) they showed that riboflavin and sodium glutarate were probably the substances in the extract responsible for the stimulation.

The general conclusions from these data are that a specific pre-sporulation nutrient is essential to abundant sporulation. If this nutrient is satisfactory and the sugar content of the medium low enough, sporulation may occur even on the agar slant, as in GORODKOWA'S or MRAK'S media. Generally, the yeast should be removed from the substrate, especially if its sugar content is high, and placed on gypsum. The water saturating the gypsum slant should be acid and should contain by-products of yeast growth and possibly some substances produced by the death and disintegration of yeast cells.

Genetical analysis requires an abundance of large viable 4-spored asci, and the present paper reports an investigation of the conditions controlling sporulation in *Saccharomyces cerevisiae*. The objective was to develop an optimal medium for the production of spores, and especially to reduce the time required to obtain them. Grape juice was found a satisfactory pre-sporulation nutrient for some yeasts but was ineffective with others. GORODKOWA'S medium and carrot agar were ineffective generally. Yeasts vary extremely in their capacity for sporulation, and it was essential to develop a medium that would activate even the poorest sporulating cultures. The study of pre-sporulation nutrition was extended and an extremely effective combination of nutrients developed. There seems to be a general correlation between the size of the ascospores and their vigor, and media which produce the larger spores were favored. Since the larger asci are easier to dissect, this characteristic has a twofold value. Immature spores are usually non-acidfast, and components of the media which reduce the number of such spores to a minimum have been selected. Throughout this work counts have been made or percentages of sporulation estimated on the slides after staining, but different

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numbers of strains as well as different strains were tested on the various media and changes in technique developed as the work progressed, so that comparisons are confined to each particular batch. For example, the designation ++++, in the earlier tests on grape-apricot-apple mixtures, does not have the same significance as it has in the later tests. In the earlier tests 4-spored asci appeared only rarely, and presence or absence was a sufficient means of distinction. As the media improved, the numbers of 4-spored asci increased, until finally media were judged on their ability to produce large 4-spored asci and on their comparative percentages. In any event, the percentage always played an important part in the selection of media. Sporulation was more abundant directly on the slants of simple potato agar than on most of the media tested, but this medium was not used because the asci were small and predominantly 2-spored, even when cultures genetically capable of producing 4-spored asci were used. Cells grown on a substrate containing sugar became much larger than those grown on a vegetable slant. Sporulation does not occur on a rich sugar medium, and therefore transfer to gypsum is required. However, since this extra step results in the production of larger spores, the advantage more than counterbalances the extra work.

## Investigation

### Grape-apricot-apple mixtures

Since Baltatu showed that grape juice was an effective pre-sporulation nutrient, it seemed probable that other fruits on which yeast occurs naturally might also be effective. The most notable of these is the apricot.

The effect on sporulation of various combinations of (*a*) bottled grape juice, (*b*) canned apricot juice, and (*c*) commercial bottled apple cider was tested. The fluids were each diluted with equal quantities of water and made into agar medium by adding 3% of agar and neutralizing with 1% of powdered $CaCO_3$. The combinations were made by mixing equal parts of the agars. Slants of the media were prepared, without filtering, and inoculated with yeast. After 2 days they were transferred to gypsum slants, and several days later slides were prepared and the number of spores estimated. The average production of spores for three strains of yeast is as indicated:

| | |
|---|---|
| Grape | + |
| Apricot | ++ |
| Apple | + |
| Apricot-apple | + |
| Grape-apple | + |
| Grape-apple-apricot | + |
| Grape-apricot | ++++* |

* 4-spored asci present.

Commercial apple cider apparently suppresses sporulation, and a mixture of grape and apricot is much more effective than either alone. The cells from the grape-apricot mixture produced the only 4-spored asci found.

### Tomato-prune-potato mixtures

The effect on sporulation of combining the grape-apricot medium with (*a*) canned tomato juice, (*b*) juice from canned prunes, (*c*) juice from canned figs, and (*d*) juice from canned potatoes was tested. The spores produced on the simple tomato medium were obviously aborted. They were tiny and were suspended in a blue-stained epiplasm. None of these combinations was as good as the grape-apricot mixture, with the possible exception of potato. Malt extract was also found to be ineffective by itself or in combination.

### Vegetable-fruit mixtures

Tests using Mrak's medium containing carrot, cucumber, beet, and potato showed it to be effective in inducing some strains to sporulate but relatively ineffective with others. Media were made combining cucumber, beet (roots and stems), and potato. About 200 gm. of each were ground in a meat grinder, boiled, and squeezed out through cheesecloth. Equal portions of each of these extracts and grape-apricot juice were used in the agar. Carrot was not used, since it had not been found useful in our earlier work. Mrak obtained spores directly on the agar slants without transfer to gypsum. The following list shows the average sporulation which occurred on the slants of these media after 7 days with five different yeast strains:

| | |
|---|---|
| Grape-apricot | + |
| Grape-apricot-cucumber | + |
| Grape-apricot-beet | + |
| Grape-apricot-potato | + |
| Grape-apricot-beet-potato | ++ |
| Grape-apricot-cucumber-potato | ++ |
| Grape-apricot-cucumber-beet | ++ |
| Grape-apricot-cucumber-beet-potato | ++++* |
| Beet | +++* |
| Potato | ++++ |
| Cucumber-beet | ++ |
| Cucumber-potato | + |
| Beet-potato | + |
| Cucumber-beet-potato | + |

* 4-spored asci present.

The media containing grape-apricot juices produced fewer spores on the slants than the vegetable media alone or in combination. The fruit media are relatively sweet and the cells on these media are large, with a large central vacuole. This agrees with Gorodkowa's findings that a decrease in sugar is essential to spore formation directly on the slant. The cells on the vegetable media are medium-sized and densely staining. The spores on the vegetable media were all acidfast, but many of them on the slants containing grape-apricot juice were non-acidfast. The most abundant production of spores occurred on potato agar, but the asci were almost exclusively 2-spored. Cucumber-beet-potato medium is the poorest. The beet extract is the most potent producer of 4-spored asci. No culture failed to produce 4-spored asci on beet agar.

As the cultures age the number of spores on the vegetable media do not increase materially, but there is a slow increase in the number when the richer grape-apricot mixtures become exhausted. Sporulation of the large vacuolate cells on the grape-apricot combination proceeds rapidly after transfer to a gypsum slant, but such transfer does not materially increase the number of spores on the single vegetable media. Spores are produced on the vegetable media as soon as growth is well started. As more components are added to the grape-apricot mixture, more spores are produced. The 5-component mixture is the best, especially when judged by the number of 4-spored asci. This may be due to the dilution of the sugar by the addition of the vegetable extracts. On the slants containing grape and apricot, spores appear slowly or not at all. The simple grape-apricot combination produces spores only after transfer to gypsum.

### Glycerin-beet mixtures

Because the spores on the grape-apricot combinations were often non-acidfast, the effect of glycerin was tested. The vegetable most conspicuously effective in producing 4-spored asci was the beet. A beet agar was made up by using 450 gm. of beet roots and 150 gm. of beet leaves; 1500 cc. of water was added and the mixture heated in the autoclave

and squeezed through cheesecloth. Half of this was made into 3% agar without glycerin and half into 3% agar with 3% glycerin. Slants were inoculated with various yeasts and cells stained after 7 days on the slants, without transfer to gypsum. The addition of glycerin inhibited sporulation on the beet-agar slant, but when the beet-glycerin cultures were transferred to gypsum, sporulation on the gypsum increased markedly. The cells grown on the beet-glycerin agar slants were large, with a big central vacuole similar to that of those grown on the media containing grape-apricot. In both cases the explanation is probably the same—sugar and glycerin increase growth on the slant but inhibit sporulation. Twenty-two strains were tested:

| | |
|---|---|
| Beet agar | ++ |
| Beet-glycerin agar | + |
| Beet-glycerin agar to gypsum | +++ |

Cow's milk and egg yolk, both used in culturing acidfast bacteria, proved ineffective in increasing sporulation.

An agar with a base of beet roots and leaves containing 3% glycerin was compared with the grape-apricot-vegetable-glycerin medium. Sixteen strains were tested. Both media were equally effective for the production of 4-spored asci, but the spores from the beet-glycerin agar were larger.

NICKERSON and THIMANN'S observation, and a study of the yeast colony by LINDEGREN and HAMILTON (9), suggested that yeast extract might be an effective agent in ascospore production. The addition of 2% dried brewers' yeast proved extremely effective in increasing both the percentage of sporulation and the number of 4-spored asci. It was also found that a relatively high concentration of grape and apricot juice in the medium produced optimal sporulation, with the proportion of 20% grape juice and 40% canned apricot nectar giving the best results.

### FINAL MEDIA AND METHODS

The following pre-sporulation medium was finally chosen:

| | |
|---|---|
| Beet (leaves) extract | 10 cc. |
| Beet (roots) extract | 20 cc. |
| Apricot juice | 35 cc. |
| Grape juice | 16.5 cc. |
| Yeast (dried) | 2 gm. |
| Glycerin | 2.5 cc. |
| Agar | 3 gm. |
| $CaCO_3$ | 1 gm. |

Water was added to give a final volume of 100 cc. The mixture was steamed for 10 minutes and tubed. Tubes were sterilized at 15 lb. for 20 minutes and slanted. Most strains of yeast will produce spores directly on the slants if allowed to grow for a few weeks. However, if spores are needed sooner, transfer to gypsum is necessary.

ENGEL'S gypsum block method was replaced by the method of GRAHAM and HASTINGS. A mixture of plaster of Paris and water (100 gm. of each) was poured into test tubes and solidified in a slanting position. These slants were dried at 50° C. for 24 hours and autoclaved.

TECHNIQUE.—About 1 cc. of sterilized water is poured over a 3-day growth of yeast on the pre-sporulation medium and allowed to stand 10 minutes, then a thick suspension is made by stirring the yeast cells around in this supernatant water. The yeast suspension is taken up in a pipette and poured over the upper part of the gypsum slant. About 3 cc. of sterile water containing enough acetic acid to bring the pH to 4 is pipetted into the lower half of the gypsum slant. The inoculated gypsum slants are incubated 1–2 days at 25° C.

ACIDFAST SPORE STAIN.—The asci are

smeared on the slide from the gypsum slope. The smear is fixed by heat and placed in a Coplin jar of carbol fuchsin for 20 minutes or longer. Destaining is accomplished by washing rapidly in 30% acetic acid while holding the slide with a forceps. The slide is not rinsed in water before destaining, and this prevents the formation of the precipitate that usually occurs if it is transferred directly to water from carbol fuchsin. Two or three rapid swishes through the acetic acid should immediately be followed by a rinse in water. If the slide is held too long in the acid, or not rinsed quickly enough in water, it will be completely destained. The success of this method depends on critical control of destaining. After the water rinse, methylene blue is used as a counterstain. This procedure stains the spores red and the vegetative cells blue.

DELAFIELD SPORE STAIN.—A simpler method is to place a tiny drop of cell suspension on a slide, add a small drop of Delafield's haematoxylin, and cover with a coverglass. This makes a permanent preparation, because the Delafield's contains glycerin and the preparation does not dry out. The cell walls of the vegetative cells and spores are clearly defined against a dark background. Rather good cytological results can be obtained by mixing 7 parts of Delafield's with 3 parts of acetic and using the same procedure. These preparations attain an optimum after about 3 days.

## SPORULATION IN SINGLE ASCUS AND SINGLE ASCOSPORE CULTURES

Data (**8, 11, 17, 18, 21**) have already been presented showing that *Saccharomyces cerevisiae* is heterothallic, and that legitimately diploid cultures almost always produce viable 4-spored asci, while single ascospore cultures only rarely do so. The cultures produced by the germination of intact 4-spored asci are usually produced by copulation of genetically different gametes and are therefore heterozygous. The homozygous or haploid cultures produced by single ascospores, when they can be induced to sporulate, produce spores of low viability (fig. 1).

Cultures originating from (*a*) vegetative cells, (*b*) single ascospores dissected from 4-spored asci, (*c*) intact 4-spored asci, and (*d*) 1-spored asci were critically examined in an analysis of four inbred generations of a yeast family, the "D" family (fig. 2). The individual cultures in the pedigree are designated as follows: Arabic numerals indicate asci; capital letters designate the ascospores isolated from asci. For example, D1A is the culture from ascospore A isolated from ascus D1. Four spores were dissected from each 4-spored ascus; in ascus D1 only two spores (D1A and D1B) produced viable colonies. D1C and D1D died and are not listed in the pedigree. Only one spore, D2B, from ascus D2 produced a viable colony. Later investigations showed that single ascospore cultures may be either haploid or illegitimately diploid. However, data concerning the genetic composition of the single ascospore cultures in this pedigree are not available.

Small Roman letters designate subcultures arising from individual colonies obtained by sowing a suspension of cells on an agar plate. For example, the original D culture was plated on agar and fourteen colonies selected. The yield of each subculture was measured in hundredths of a cubic centimeter per 10 cc. of medium, according to the technique already described (**10**). All fourteen subcultures yielded more than 6.0.

The original D culture was also placed on gypsum, and two 4-spored and three 1-spored asci were isolated. The two 4-spored asci were dissected and the spores planted separately in culture tubes. Only three of the ascospores from the 4-spored asci produced cultures, while all three 1-spored asci germinated and each produced a viable culture. The yield of the cultures originating from the 1-spored asci approximated that of the single colonies selected by plating vegetative cells. This means that 1-spored asci are genetically similar to the vegetative cells from which they arise. The yield of the single ascospore cultures from 4-spored asci is variable, indicating that the original culture is heterozygous for the factors affecting yield.

The second generation originated from culture Db, which gave a high yield and produced viable 4-spored asci on gypsum. Only one of eight 1-spored asci failed to germinate, and the yield of the 1-spored ascus cultures compared favorably with that of the cultures from which they originated. Fourteen 4-spored asci were dissected and the fifty-six single spores isolated; thirty-three spores produced viable cultures. Both yield and ascospore production were rather variable among these cultures. Only two of the thirty-three cultures produced 4-spored asci; the others generally produced 1- or 2-spored asci.

Sixteen intact 4-spored asci were isolated and planted in hanging drops. All germinated to produce cultures. The resulting colony was suspended in water and plated on agar. Individual colonies were selected and tested for yield. Although only 19% of single ascospore cultures gave a yield of 6.0 or more, 45% of the cultures from self-fertilized asci fell in this category. The following shows the data:

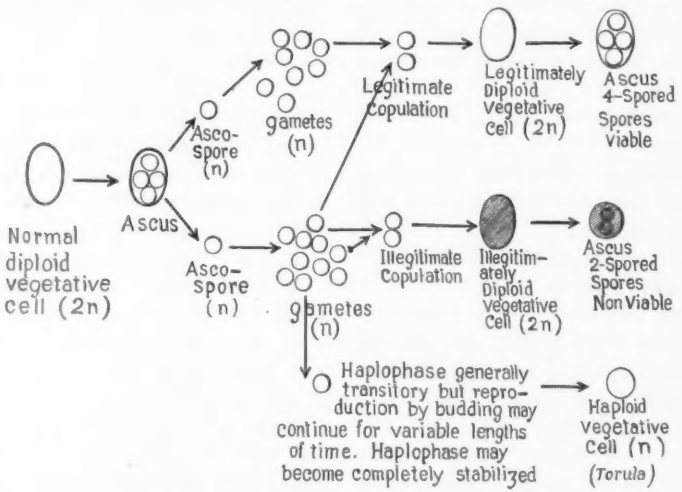
Normal diploid vegetative cell (2n)
Ascus
Asco-spore (n)
Asco-spore (n)
gametes (n)
gametes (n)
Legitimate Copulation
Legitimately Diploid Vegetative Cell (2n)
Ascus 4-Spored Spores Viable
Illegitimate Copulation
Illegitimately Diploid Vegetative Cell (2n)
Ascus 2-Spored Spores Non Viable
Haplophase generally transitory but reproduction by budding may continue for variable lengths of time. Haplophase may become completely stabilized
Haploid Vegetative Cell (n)
(Torula)

FIG. 1.—Haplophase and diplophase in *Saccharomyces cerevisiae*

| CLASS RANGE | SINGLE ASCOSPORE CULTURES | COLONIES FROM SELF-FERTILIZED ASCI |
|---|---|---|
| 4.0–4.4 | 6 | 1 |
| 4.5–4.9 | 10 | 3 |
| 5.0–5.4 | 23 | 17 |
| 5.5–5.9 | 42 | 34 |
| 6.0–6.4 | 16 | 34 |
| 6.5–6.9 | 3 | 11 |

The highest-yielding colonies from the self-fertilized asci were placed on gypsum, and most of them produced an abundance of spores. Three of them (Db5d, Db5h, and Db51) produced viable spores and were used to propagate the third generation.

Only two of the single ascospore cultures in the second generation (Db1A and Db2A) produced 4-spored asci. A total of five 4-spored asci from these cultures were isolated but no cultures were obtained. Sixteen 1-spored, eleven 2-spored, and four 3-spored asci also failed to produce cultures. Some of the asci from single ascospore culture Db25B were viable.

Only twelve cultures were obtained from ninety-four spores originating from single ascospore cultures. In contrast, thirty-five cultures were obtained from seventy-one ascospores isolated from cultures arising from self-fertilized asci. Many unpublished corroborative data have now been accumulated, and the difference between the viabilities of spores arising from single ascospores and from intact asci is much greater than these early data indicate.

One of the self-fertilized asci from culture Db5h (Db5h5b) produced viable 4-spored asci and was carried into the fourth generation. Culture Db512a also produced viable 4-spored asci and was propagated into the fourth generation.

In the pedigree of the D family the only vigorously viable 4-spored asci were obtained from those self-fertilized, and cultures from this source were generally more vigorous as to yield and ascospore viability than cultures from single ascospores. On this basis it seems possible to distinguish two kinds of yeast cultures, those arising from single ascospores and those arising from single asci. This observation has proved useful in the analysis of various commercial baking yeasts.

## Sporulation of commercial baking yeasts

Table 1 shows percentages of sporulation and the types of asci found in forty baking yeasts which had been grown on grape-apricot medium and transferred to gypsum slants. The cultures designated

TABLE 1

Variation in ascospore production of commercial yeasts

| Serial no. | Cells sporulating (%) | Type of ascus | Remarks |
|---|---|---|---|
| B.* | 0.5 | 1, 2 | |
| LR | 5.0 | 1, 2, 3 | |
| NG4A | 0.5 | 1, 2 | |
| R | 0.1 | 1, 2 | |
| 379 | 2.0 | 1, 2 | |
| 795 | 0.1 | 1, 2 | |
| 796 | 5.0 | 1, 2 | |
| 797 | 2.0 | 1, 2 | |
| 798 | 1.1 | 1, 2 | |
| 805 | Very rare | 1, 2 | |
| 807 | 0.5 | 1, 2 | Many 3's |
| 808 | 5.0 | 1, 2, 3 | |
| 809 | 0.0 | ..... | |
| 810 | 0.0 | ..... | |
| 811 | 5.0 | 1, 2 | |
| 813 | 1.0 | 1, 2 | |
| 814 | 10.0 | 1, 2 | |
| 815 | 5.0 | 1, 2 | |
| 817 | 15.0 | 1, 2 | |
| 818 | 0.3 | 1, 2, 3 | Mostly 3's |
| 822 | 0.0 | ..... | |
| 823 | 5.0 | 1, 2, 3 | |
| 824 | 0.01 | 1 | |
| 826 | Very rare | 1, 2 | |
| BE | 5.0 | 1, 2, 3, 4 | Few 4's |
| D | 25.0 | 1, 2, 3, 4 | 5% 4's |
| F | 50.0 | 1, 2, 3, 4 | 5% 4's |
| G | 30.0 | 1, 2, 3, 4 | 20% 4's |
| HD | 10.0 | 1, 2, 3, 4 | 10% 4's |
| LM | 40.0 | 1, 2, 3, 4 | 5% 4's |
| M | 30.0 | 1, 2, 3, 4 | 5% 4's |
| 794 | 30.0 | 2, 3, 4 | Some 4's |
| 799 | 20.0 | 2, 3, 4 | |
| 800 | 30.0 | 2, 3, 4 | Some 4's |
| 801 | 5.0 | 1, 2, 3, 4 | |
| 806 | 10.0 | 2, 3, 4 | |
| 812 | 20.0 | 2, 3, 4 | Some 4's |
| 816 | 12.0 | 1, 2, 4 | |
| 820 | 12.0 | 1, 2, 3, 4 | |
| 825 | 7.0 | 1, 2, 3, 4 | |

# FIRST GENERATION

Dissected 4-Spored asci

| | Yield |
|---|---|
| D1A | 4.0 |
| D1B | 1.3 |
| D2B | 5.5 |

1-Spored asci

| | Yield |
|---|---|
| D3A | 6.7 |
| D4A | 6.0 |
| D5A | 6.0 |

Single colonies

| | class range 6.0/6.4 | 6.5/6.9 | 7.0/7.4 |
|---|---|---|---|
| Da to Dn | 3 | 10 | 1 |

Selected colonies

| | Yield |
|---|---|
| Db | 6.8 |
| Df | 6.5 |
| Dm | 6.8 |

# SECOND GENERATION

1-Spored asci

| | Yield |
|---|---|
| Db33A | 5.5 |
| Db34A | 6.2 |
| Db35A | 6.9 |
| Db36A | n.g |
| Db38A | 6.7 |
| Db39A | 6.8 |
| Db40A | 6.3 |
| Db41A | 6.2 |

4-Spored asci dissected

| | Yield | Spores |
|---|---|---|
| Db1A | 6.5 | 15% |
| Db1B | 6.0 | 15% |
| Db1C | 4.5 | 0 |
| Db2A | 4.1 | 5% |
| Db2B | 6.0 | 5% |
| Db3A | 4.0 | |
| Db17A | 5.5 | 40% |
| Db17B | 5.0 | rare |
| Db17C | 5.8 | 5% |
| Db17D | 5.5 | 5% |
| Db18A | 5.5 | 0 |
| Db19A | 6.0 | 50% |
| Db19B | 5.8 | 50% |
| Db19C | 5.9 | 30% |
| Db19D | 5.9 | rare |
| Db20A | 5.8 | 70% |
| Db20B | 5.1 | 2% |
| Db20C | 5.8 | 10% |
| Db21A | 4.8 | |
| Db21B | 4.8 | |
| Db21C | 5.8 | 40% |
| Db21D | 5.1 | 5% |
| Db22C | 5.2 | 0 |
| Db23A | 5.0 | 20% |
| Db23B | 5.3 | 20% |
| Db23C | 5.8 | 30% |
| Db23D | 5.9 | 20% |
| Db25B | 6.4 | 60% |
| Db25C | 5.3 | rare |
| Db25D | 6.0 | 40% |
| Db26C | 6.0 | |
| Db37A | 5.4 | rare |
| Db37B | 5.6 | rare |
| Db24 | No spores germinated | |

4-Spored asci Selfed

| Ascus | Colonies | class range 4.0/4.4 | 4.5/4.9 | 5.0/5.4 | 5.5/5.9 | 6.0/6.4 | 6.5/6.9 |
|---|---|---|---|---|---|---|---|
| Db5 | a-i | | | 1 | 1 | | 3 |
| Db6 | a-j | | | 5 | 5 | | |
| Db7 | a-i | | | 1 | 7 | 1 | |
| Db8 | a-h | | | 2 | 4 | 2 | |
| Db9 | a-h | | | 4 | 2 | 2 | |
| Db10 | a-g | | | | 1 | 5 | |
| Db11 | a-h | | 1 | | | 7 | |
| Db12 | a-h | | 1 | 2 | 3 | 2 | |
| Db13 | a-g | | | | 4 | 3 | |
| Db14 | a-h | | | 2 | 1 | 4 | 1 |
| Db15 | a-h | | | 1 | 2 | 5 | |
| Db16 | a-h | 1 | | | 2 | 3 | 1 |
| Db28 | a-p | | | 1 | 5 | 4 | 6 |
| Db29 | a-s | | | 3 | 6 | 2 | 3 |
| Db30 | a-n | | 2 | 1 | 7 | 4 | |
| Db31 | a-s | | 1 | 2 | 3 | 7 | 3 |

Selected Colonies

| | Yield | Spore | Remarks |
|---|---|---|---|
| Db5d | 6.7 | 80% | viable spores |
| Db5h | 6.8 | 70% | viable spores |
| Db5i | 6.5 | 70% | viable spores |
| Db11d | 6.6 | 50% | small spores |
| Db14a | 6.5 | 1% | |
| Db28h | 6.5 | 50% | spores non-viable |
| Db29k | 6.9 | 60% | spores poorly viable |
| Db29l | 6.5 | 40% | |
| Db31b | 6.9 | 50% | |
| Db31i | 6.9 | 70% | |
| Db31K | 6.7 | 95% | spores non-viable |

FIG. 2

# THIRD GENERATION

## Db5d

4-Spored Asci Dissected

| | | |
|---|---|---|
| Db5d1A | 5.5 | gray |
| Db5d1B | 5.6 | gray |
| Db5d1C | 5.4 | gray |
| Db5d14 | no spores | germinated |
| Db5d15 | no spores | germinated |
| Db5d16A | 4.5 | gray |
| Db5d16B | 5.4 | gray |
| Db5d17A | 4.5 | gray |
| Db5d18A | 4.5 | gray |
| Db5d18B | 5.8 | gray |
| Db5d18C | 4.7 | gray |
| Db5d18D | lost | ---- |
| Db5d19A | 5.0 | gray |
| Db5d19B | lost | ---- |
| Db5d21A | 5.5 | gray |
| Db5d21B | 5.1 | gray |
| Db5d21C | 5.7 | gray |

12 4-spored asci selfed and formed colonies
Discarded because of gray color

### Db1A

| | | |
|---|---|---|
| 12 | 1-spored asci | n.g |
| 1 | 4-spored asci | n.g |

### Db2A

| | | |
|---|---|---|
| 4 | 1-spored asci | n.g |
| 11 | 2-spored asci | n.g |
| 4 | 3-spored asci | n.g |
| 4 | 4-spored asci | n.g |

### Db25B

| | | |
|---|---|---|
| 4 | 3-spored asci selfed germinated | |
| 4 | 3-spored asci dissected | n.g |

## Db5h

4-Spored Asci Dissected

| | | |
|---|---|---|
| Db5h1A | 5.7 | |
| Db5h1B | 5.6 | |
| Db5h6A | 5.6 | |
| Db5h6B | 6.2 | |
| Db5h6C | 3.2 | dark |
| Db5h7A | 5.6 | |
| Db5h7B | 5.0 | |
| Db5h7C | 3.5 | |
| Db5h8A | 5.4 | |
| Db5h8B | 5.0 | gray rough |
| Db5h9A | 5.4 | |
| Db5h9B | 5.0 | gray rough |
| Db5h10A | 5.6 | |
| Db5h11A | 6.3 | |

4-Spored Asci Selfed

| Ascus | Colonies | Class Range 5.5/5.9 | 6.0/6.4 | 6.5/7.0 | 7.5/8.0 |
|---|---|---|---|---|---|
| Db5h2 | a-n | 1 | 10 | 3 | |
| Db5h3 | a-n | 5 | 9 | | |
| Db5h4 | a-p | | 12 | 4 | |
| Db5h5 | a-o | | | 9 | 7 1 |

Colonies Selected

| | Yield | spores | selected | Remarks |
|---|---|---|---|---|
| Db5h3c | 6.0 | 20% | 1, 2 | |
| Db5h3e | 6.0 | 20% | 1, 2 | |
| Db5h3j | 6.0 | 80% | 1, 2 | |
| Db5h4c | 6.8 | 3% | 1, 2 | Small |
| Db5h4d | 6.3 | 10% | 1, 2, 3 | Small |
| Db5h4j | 6.6 | 30% | 1, 2, 3 | Small |
| Db5h4o | 6.5 | 20% | | Small |
| Db5h5b | 6.8 | 50% | | many 4's |
| Db5h5g | 7.0 | 70% | 1, 2 | |
| Db5h5i | 6.8 | 50% | 1, 2, 3 | |
| Db5h5m | 6.8 | 70% | 2, 3 | |
| Db5h5o | 7.6 | 50% | 2, 3 | |

## Db5i

4-Spored Asci Dissected
Db5i1A germinated

Random Ascospores from ruptured asci

| | Yield | |
|---|---|---|
| Db5i6E | 5.5 | |
| Db5i6F | 5.5 | |
| Db5i6G | 5.0 | |
| Db5i6H | 5.0 | |
| Db5i6I | 6.2 | |
| Db5i6J | 5.6 | |
| Db5i6K | 4.0 | gray |
| Db5i6L | n.g | |
| Db5i6M | n.g | |
| Db5i6N | n.g | |
| Db5i6O | n.g | |
| Db5i6P | n.g | |

4-Spored Asci Selfed

| Ascus | Colonies | Class Range 5.0/5.4 | 5.5/5.9 | 6.0/6.4 | 6.5/6.9 | 7.0/7.4 |
|---|---|---|---|---|---|---|
| Db5i2 | a-l | | | | 2 | 10 |
| Db5i3 | a-o | 3 | 1 | 8 | 3 | |
| Db5i4 | a-h | | 2 | 10 | | |
| Db5i5 | a-m | 1 | 9 | 2 | | |

Colonies Selected

| | Yield | spores |
|---|---|---|
| Db5i2a | 50% | misshapen |
| Db5i2i | 50% | misshapen |
| Db5i2j | 50% | 3's only |

# FOURTH GENERATION

## Db5h5b

4-spored asci dissected

| Spore | Yield |
|---|---|
| Db5h5b1A | 5.0 |
| Db5h5b1B | 5.0 |
| Db5h5b1A | 5.3 |
| Db5h5b2B | 5.6 |
| Db5h5b2C | 5.9 |
| Db5h5b2A | 5.1 |
| Db5h5b3A | 5.3 |
| Db5h5b4B | 5.5 |
| Db5h5b4C | 5.5 |

| Ascus | Colonies |
|---|---|
| Db5h5b5 | a-i |
| Db5h5b8 | a-j |
| Db5h5b11 | |
| Db5h5b12 | |
| Db5h5b6 | all germinated. No selections made |
| Db5h5b7 | |
| Db5h5b9 | |
| Db5h5b10 | |

4-spored asci selfed

| Class Range 4.0/4.4 | 4.5/4.9 | 5.0/5.4 | 5.5/5.9 | 6.0/6.4 | 6.5/6.9 | 7.0/7.5 |
|---|---|---|---|---|---|---|
| | | | 3 | 6 | 1 | |
| | 1 | | 4 | 4 | | |
| | | | 1 | 3 | 1 | 1 |
| | | 4 | 2 | | | |

## Db5i2a

4-spored asci dissected

| | Yield |
|---|---|
| Db5i2a1A | 5.0 |
| Db5i2a1B | 5.5 |
| Db5i2a1C | 5.3 |
| Db5i2a2A | 5.0 |
| Db5i2a2B | 5.0 |
| Db5i2a2C | 5.0 |
| Db5i2a2D | 5.2 |

4-spored asci selfed

Db5i2a3, Db5i2a4, Db5i2a5, Db5i2a6 — all germinated. No selections made

FIG. 2.—*Continued*

by letter are commercial bakers' yeasts collected on the market and those designated by number are commercial bakers' yeast cultures obtained from Dr. L. J. WICKERHAM of the Northern Regional Laboratory of the U.S.D.A. at Peoria, Illinois. The percentages of cells which sporulated were usually estimated by inspection. Only sixteen cultures contained 4-spored asci, and only in the most favorable conditions were as many as 5 or 6% of the vegetative cells transformed into 4-spored asci.

Numerous duplicate tests indicate that, in spite of the considerable variation encountered, it is fairly easy to distinguish cultures which sporulate well from those which sporulate poorly. Generally 4-spored asci are found only in those cultures in which almost 20% of the vegetative cells are transformed into asci. In all cultures there were more asci in the 2-spored category than in any other.

It is clear that the cultures fall into two categories, (*a*) those which sporulate poorly and do not produce 4-spored asci and (*b*) those which sporulate relatively well and produce 4-spored asci. On the basis of the data obtained in the pedigree of the D family, the conclusion that the former cultures originated from single ascospores and the latter from self-fertilized asci seems inescapable.

## SPORULATION OF R-STRAIN SUBCULTURES

Thirty-one subcultures were obtained by plating out the R-strain culture, and each was tested for ascospore production (table 2). They all fall into the poorly sporulating category, with only a few rare 4-spored asci. Subcultures of a poorly sporulating culture rarely show improvement over the parent strain, but it is clear from the pedigree of the D family that subcultures of a vigorously sporulating culture are often extremely inferior to the parent in spore-producing ability.

TABLE 2

SPORULATION IN CULTURES ISOLATED FROM R STRAIN

| Serial no. | Cells sporulating (%) | Type of ascus | Remarks |
|---|---|---|---|
| Ra | 2.0 | 1, 2 | |
| Rb | 1.0 | 1, 2 | |
| Rc | 0.5 | 1 | |
| Rd | 0.5 | 2, 3 | Small |
| Re | 3.0 | 1, 2, 3 | |
| Rf | 5.0 | 1, 2 | |
| Rg | 0.5 | 1, 2 | |
| Rh | 0.5 | 1, 2 | Small |
| Ri | 1.0 | 1, 2, 3 | Small |
| Rj | 0.5 | 1, 2 | |
| Rk | 0.5 | 1, 2 | |
| Rl | 0.5 | 1, 2 | |
| Rm | 5.0 | 1, 2, 3 | Large |
| Rn | 0.5 | 1, 2 | |
| Ro | 1.0 | 1, 2 | |
| Rp | 1.0 | 1, 2 | |
| Rq | 2.0 | 1, 2, 3 | |
| Rr | 5.0 | 1, 2, 3, 4 | Large |
| Rs | 5.0 | 1, 2 | |
| Rt | 1.0 | 1, 2 | |
| Ru | Rare | 1, 2 | |
| Rv | 2.0 | 1, 2 | |
| Rw | 3.0 | 1, 2 | |
| Rx | 3.0 | 1, 2 | Small, irregular |
| Ry | 0.5 | 1, 2 | Small, irregular |
| Rz | 7.0 | 2, 3 | Large spores |
| Raa | 1.0 | 2, 3, 4 | |
| Rba | 5.0 | 1, 2, 3 | |
| Rca | 2.0 | 1, 2 | |
| Rda | 2.0 | 1, 2, 3, 4 | |
| Rea | 2.0 | 1, 2, 3, 4 | |

Individual 2-, 3-, or 4-spored asci from various R subcultures were selected and planted on hanging drops of agar in a moist chamber with a micromanipulator. It was necessary to search diligently to obtain any 3- or 4-spored asci, although in some cultures 1- and 2-spored ones were rather easy to find. Most of the spores failed to germinate. Some of them germinated but produced only a small

cluster of the round haploid cells, and these all died. Transfer to a fresh culture tube failed to produce a colony. Only one viable colony was obtained from forty-seven 2-spored asci.

TABLE 3

NUMBER OF VIABLE COLONIES OBTAINED FROM 1-, 2-, 3-, AND 4-SPORED ASCI OF CULTURES ORIGINATING FROM R STRAIN

| Subculture | Type of ascus | No. of asci | Viable colonies |
|---|---|---|---|
| Rda | 1 | 4 | 3 |
| Rda | 2 | 4 | 0 |
| Rda | 4 | 1 | 0 |
| Raa | 1 | 8 | 4 |
| Rz | 2 | 4 | 0 |
| Rz | 3 | 4 | 1 |
| Rea | 1 | 4 | 3 |
| Rea | 2 | 33 | 1 |
| Rea | 4 | 1 | 0 |
| Rt | 2 | 6 | 0 |
| Rt | 4 | 1 | 0 |

In contrast to the 2-, 3-, and 4-spored asci, the 1-spored were much more viable and germinated differently. The viable spores from 1-spored asci germinated directly to produce large ellipsoidal diploid cells, instead of the small round haploid cells characteristic of the ascospores from the 2-, 3-, and 4-spored asci. Ten of sixteen spores from 1-spored asci produced living colonies (table 3).

The R-strain culture obviously has all the characteristics of a single ascospore culture—poor sporulation, low viability, and predominantly 2-spored asci. Diligent efforts to select a subculture with a superior ability to produce spores yielded only negative results.

## Summary

1. Sporulation in *Saccharomyces cerevisiae* is influenced by the genetic constitution of the culture as well as by the composition of the nutrient medium. A specific nutrient medium which gives maximum sporulation has been developed and described.

2. On this medium, heterozygous, legitimately diploid cultures produce an abundance of viable 4-spored asci. Single ascospore cultures (which may be either illegitimately diploid or haploid) sporulate much more irregularly; some do not sporulate at all. The asci from single ascospore cultures generally contain only one or two spores, and their viability is much diminished.

3. A survey of forty commercial baking yeasts was made. Some were legitimately diploid, while others were of single ascospore origin.

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## LITERATURE CITED

1. BALTATU, E., Mycoderma als echte *Saccharomycetes*. Cent. Bakt. II. **101**:196–225. 1939.
2. BEYERINCK, M. W., Schizosaccharomycetes ochtosporous, eine achtsporige Alkohol hefe. Cent. Bakt. I. **16**:49–58. 1892.
3. ———, Ueber Regeneration der Sporenbildung wo diese Funktion im Verschwinden begriffen ist. Cent. Bakt. II. **4**:657–663; 721–730. 1898.
4. ENGEL, L., Les Ferments alcooliques. University of Paris. 1–59. 1872.
5. GORODKOWA, A. A., Ueber das Verfahren rasch die Sporen von Hefepilzen zu gewinnen. Bull. Jard. Imp. Bot. St. Peters. **8**:163–170. 1908.
6. GRAHAM, V. R., and HASTINGS, E. G., Studies on film-forming yeasts. Canad. Jour. Res. **19**:251–256. 1941.
7. HANSEN, E., Recherches sur la physiologie et la morphologie des ferments alcooliques. II. Les ascospores chez le genre *Saccharomyces*. Compt. Rend Lab. Carls. **2**:29–85. 1883.
8. KRUIS, K., and ŠATAVA, J. O. vývoji a klíčení spór jakož i sexualitě kvasinek. Nakl. Č., Akad. Praha. 1918.
9. LINDEGREN, C. C., and HAMILTON, ELIZABETH, Autolysis and sporulation in the yeast colony. BOT. GAZ. **105**:316–321, 1944.
10. LINDEGREN, C. C., and LINDEGREN, GERTRUDE,

Environmental and genetical variations in yield and colony size of commercial yeasts. Ann. Mo. Bot. Gard. 30:71–82. 1943.

11. ———, and ———, Selecting, inbreeding, recombining and hybridizing commercial yeasts. Jour. Bact. 46:405–419. 1943.
12. MANEVAL, W. E., A method of securing spores of yeasts. BOT. GAZ. 78:122–125. 1924.
13. MRAK, E. M., PHAFF, H. J., and DOUGLAS, H. C., A sporulation stock medium for yeasts and other fungi. Science 96:432. 1942.
14. NICKERSON, W. J., and THIMANN, K. V., The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 28:617–621. 1941.
15. ———, and ———, The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 30:94–101. 1943.
16. REES, M., Zur Naturgeschichte der Bierhefe, *Saccharomyces cerevisiae* Meyen. Bot. Zeit. 27: 106–118. 1869.
17. ŠATAVA, J., O Redukovaných Formách Kvasinek. V. Praze Nakladem Vlastnim. 1918.
18. ———, Les formes sexuelles et asexuelles des levures et leur pouroir fermentatif. III. Cong. Internat. Tech. Chim. Ind. Agr. Paris. 1934.
19. SEYNES, M. J. DE, Sur le mycoderma vini. Compt. Rend. Acad. Sci. 67:105–109. 1868.
20. WELTEN, H., Wan bildet die Hefe Sporen? Mikrokosmos 8:3–5; 41–43. 1914.
21. WINGE, O., On haplophase and diplophase in some *Saccharomyces*. Compt. Rend. Lab. Carls. Serie Physiol. 21:77–112. 1935.

# AUTOLYSIS AND SPORULATION IN THE YEAST COLONY[1]

CARL C. LINDEGREN AND ELIZABETH HAMILTON

Knowledge of life-cycles and variations in bacteria has developed almost exclusively from the study of isolated individual cells, without consideration of the structural organization of the colony. LEGROUX and MAGROU (8) studied the structure of colonies of *Vibrio cholerae* and of a number of other bacteria. They discovered that rod-shaped variants of the *Vibrio* appear in perpendicularly arranged packets on the exterior of the colonies, held together by a transparent substance apparently exuded from the cells. They assumed that this substance gave the rough colonies their rigidity. A special stain was used:

| | |
|---|---|
| Eosinate of methylene blue | 7.0 gm. |
| Eosinate of toluidin blue | 1.5 |
| Toluidin blue | 5.0 |
| Methyl alcohol (99.5%) | 490 cc. |

The rod-shaped organisms in the outer layer of the colony were made up of a violet central region and an azure exterior. The central region underwent division into two or four rounded violet particles, often of unequal size. This phenomenon was found in *Vibrio*, in the typhoid bacillus, in the diphtheria bacillus, and in the tubercle bacillus. KAHN (7), apparently unacquainted with the work of LEGROUX and MAGROU, confirmed their findings in a study of the tubercle bacillus but concluded that the outer layer was the younger part of the colony. The work of LEGROUX and MAGROU proves this view untenable.

PISOVA (18) found that, when a yeast colony grows on agar, a pseudo-mycelial growth of long fibrous cells penetrates the agar, especially at a high sugar concentration. After a few days or weeks, the surface cells begin to autolyze, continuing until an outer layer of autolyzed cells is formed.

## Experimentation

After yeast colonies had been grown on malt-yeast agar,[2] portions of the agar containing colonies were cut out and dropped into Flemming's solution. After

[1] This work was supported by a grant from Anheuser-Busch, Inc., St. Louis.

[2] Malt extract 10.0%, dextrose 0.5%, dried brewers' yeast 0.5%, agar 3.0%, $CaCO_3$ 1.0%.

24 hours the killing fluid was washed out in tap water, and the material was imbedded in paraffin. Sections were made and stained with a variety of dyes. Direct smears were also made by cutting the fresh colony in half vertically with a razor and pressing the exposed section gently against the slide. The cells were fixed on the slide by heat and stained.

Figure 1*A* shows a section of the yeast colony. The outer layer of autolyzed cells stains very lightly, and the inner central mass of vegetative cells with their dense protoplasts is much darker. A pseudomycelium of yeast cells penetrates the agar and is thickest and deepest at the edges of the colony, apparently where oxygen is most abundant. The thin peripheral film of cells at the edge of the colony spreads over the surface of the agar. Figure 1*B* shows details at the ends of the section, with cells penetrating the agar. At the points of origin of penetration the cells are very abundant. This may be due to the channeling of the substrate nutrient into these regions along the cracks made by the pseudomycelium.

Figure 2 shows the central vegetative cells, many of which are extremely small, indicating that cell division continued after the nutrients became less readily available and competition resulted in a decrease in cell size. Figure 3 shows the pseudo-mycelium produced in the agar in higher magnification.

Figures 4 and 5 illustrate the autolyzed layer containing the asci. Only a few cells produce asci. Autolysis apparently occurs early in the history of the colony, at least before competition reduces cell size. The autolyzed layer contains the only asci found, suggesting that autolysis supplies essential nutrients on which sporulation depends. NICKERSON and THIMANN (16) reported a correlation in *Zygosaccharomyces* between the number of dead cells and the number of cells copulating and proposed that "stimulating" substances were released by the dead cells which cause those still living to conjugate and to sporulate. Later (17) they showed that riboflavin and sodium glutarate were specific substances which promoted copulation and sporulation. MANEVAL (15) pointed out that spores appear on the outside of a stored yeast cake.

In some of the contact smears the autolyzed cells were not so shrunken as those obtained by the paraffin method. The walls seemed relatively intact, but there were no stainable cell contents. The "ghost" cells were larger than the densely stained cells in the vegetative section of the colony. These autolyzed cells, which apparently serve as sources of nutrients for the sporogenous cells, have a parallel in the paraphyses found in Pyrenomycetes and Discomycetes which also act as nurse cells. In some regions of the autolyzed layer, small clusters of round, apparently haploid, cells were found, suggesting that some spores may germinate in the layer. New genetic rearrangements could result from such spore germination and subsequent copulation. These experiments suggested that yeast autolysate is an essential nutrient for sporulation and led to the addition of 2% dried brewers' yeast to a pre-sporulation medium, with excellent results (14).

## Discussion

The striking parallelism between the structure of yeast colonies and those of bacteria, as shown by LEGROUX and MAGROU, suggests that the life-cycles may be similarly parallel. Yeasts possess a proved nuclear mechanism, and the vegetative cells undergo meiosis and

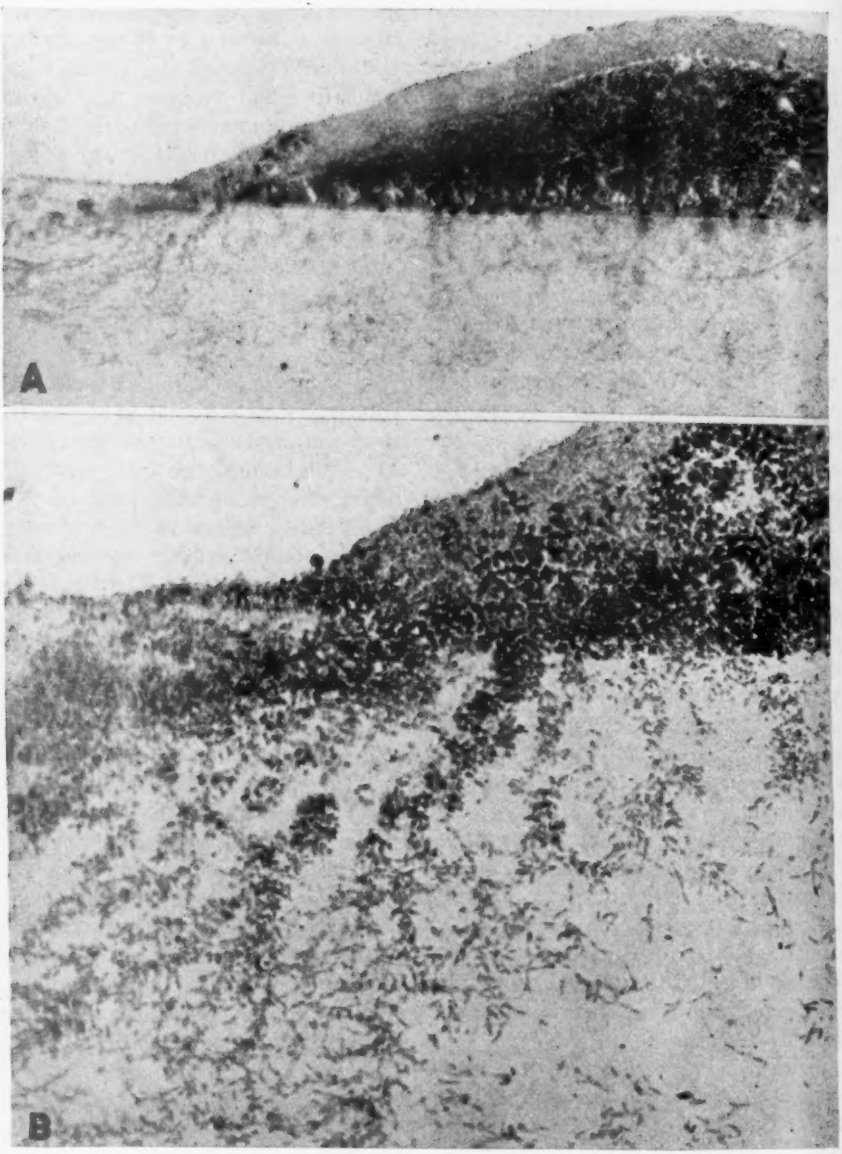
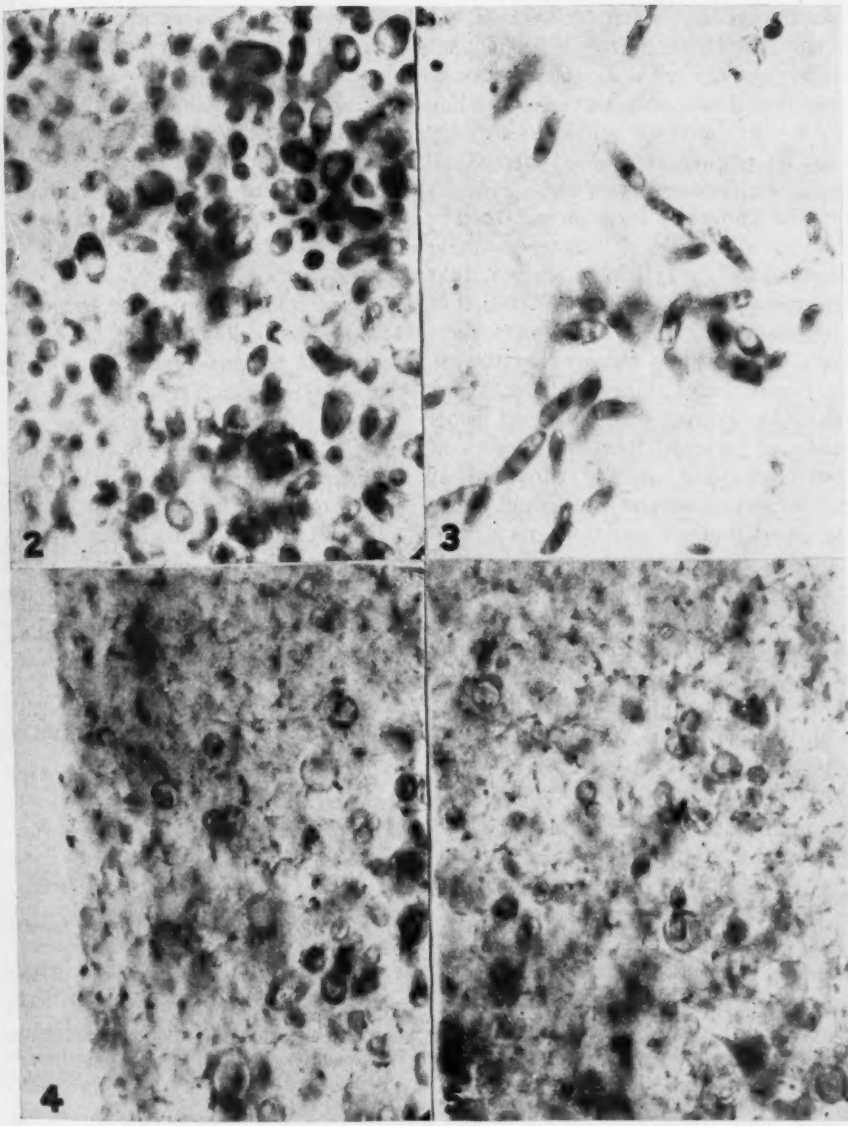



FIG. 1.—*A*, cross-section of yeast colony showing outer autolyzed layer, central region containing vegetative cells, and elongate pseudo-mycelial cells penetrating agar. *B*, left end of *A*, under higher magnification, showing penetration of agar by the pseudo-mycelium.




FIGS. 2–5.—Fig. 2, central region of vegetative cells in yeast colony. Neither spores nor autolyzed cells present but many small cells apparently produced by continued division in region of diminished nutrient. Fig. 3, higher magnification of pseudo-mycelium in agar. Figs. 4, 5, highly magnified outer layer of colony. Most cells autolyzed and walls disintegrated; those not autolyzed have sporulated and become asci. Many contain one and two spores; one 4-spored ascus visible.

sporulation in the outer layer of the colony. In bacterial colonies a similar division of the cell contents into two or four bodies occurs in the corresponding layer. The analogy suggests strongly that the granulation of the bacterial cell is the result of a reduction division similar to that known to occur in yeasts. The cytological analyses of BADIAN (1–5) and LINDEGREN (9–13) have shown that chromosomes exist in bacteria and that they undergo a series of changes comparable with those observed in the reduction divisions of higher plants. Using the same technique as developed in his study of bacteria, BADIAN (6) made a complete study of the chromosomal mechanism of several species of yeast. He found that all contained two chromosomes in the haplophase which fused end to end (presumably at the chromomeres) to produce two chromosomes in the diplophase. Reduction occurred at spore formation. Dr. HAMPTON CARSON of Washington University has confirmed the fact that yeasts contain two chromosomes in the diplophase, by using the Feulgen stain. It would seem that the work of BADIAN should supersede all earlier descriptions of chromosomes in yeast. The accuracy of his cytological findings in yeasts tends to confirm his descriptions of reduction divisions in bacteria, which suggest that spore and granule formation in the bacteria may be homologous to ascospore formation in yeasts.

## Summary

Yeast colonies contain an outer layer of autolyzed cells, and this layer is the region in which sporulation occurs. Autolysis of yeast cells probably provides a substrate favorable for sporulation. LEGROUX and MAGROU showed that in bacterial colonies a similar structural (one might almost say histological) differentiation exists. The bacterial cells in the outer layer of the colonies produced tetrads, which may possibly be homologous to the ascospores of yeasts.

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## LITERATURE CITED

1. BADIAN, J., Z Cytologji Miksobakteryj. Acta Soc. Bot. Pol. 7:55–71. 1930.
2. ———, Eine cytologische Untersuchung ueber das Chromatin und den Entwicklungszyklus der Bacterien. Ark. Mikro. 4:409–418. 1933.
3. ———, Eine cytologische Untersuchung ueber die *Mixobacterie polyangium fuscum*. Acta Soc. Bot. Pol. 10:361–378. 1933.
4. ———, Sur la Cytologie du *Bacillus megatherium*. *Ibid.* 13:69–74. 1935.
5. ———, Ueber die cytologische Structur und den Entwicklungszyklus der Actinomyceten. *Ibid.* 13:105–126. 1936.
6. ———, Sur la cytologie des levures. Bull. Acad. Pol .Sci. Lett. Ser. B. Science Nat. 50:61–87. 1937.
7. KAHN, M. C., and NONIDEZ, JOSÉ, Non-acidfast rods and granules in vertical sections of *Mycobacterium tuberculosis* colonies. Proc. Soc. Exp. Biol. Med. 30:577–582. 1933.
8. LEGROUX, RENÉ, and MAGROU, J., État organisé des colonies bacteriennes. Ann. Inst. Past. 34: 417–431. 1920.
9. LINDEGREN, C. C., and MELLON, R. R., Nuclear phenomena suggesting a sexual mechanism for the tubercle bacillus. Proc. Soc. Exp. Biol. Med. 30:110–112. 1932.
10. LINDGREN, C. C., Genetical studies of bacteria. I. The problem of the bacterial nucleus. Cent. Bakt. II. 92:41–48. 1935.
11. ———, Genetical studies of bacteria. II. The problem of bacterial variation. *Ibid.* II. 92: 113–122. 1935.
12. ———, Genetical studies of bacteria. III. Origin of G-type colonies by transgenation. *Ibid.* II. 92:389–400. 1935.

13. ———, Nuclear apparatus and sexual mechanism in a micrococcus. Iowa State Coll. Jour. Sci. 16:307–318. 1942.
14. LINDEGREN, C. C., and LINDEGREN, GERTRUDE, Sporulation in *Saccharomyces cerevisiae*. BOT. GAZ. 105:304–316. 1944.
15. MANEVAL, W. E., A method of securing spores of yeast. BOT. GAZ. 78:122–125. 1924.
16. NICKERSON, W. J., and THIMANN, K. V., The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 28:617–621. 1941.
17. ———, The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 30:94–101. 1943.
18. PISOVA, MILDRED, Anatomy of yeast colonies. Unpublished thesis. Karl University, Prague. (Additions by Dr. Němeč. 1930.)

# PHOTOPERIODICITY OF BAERIA CHRYSOSTOMA

E. SIVORI AND F. W. WENT

## Introduction

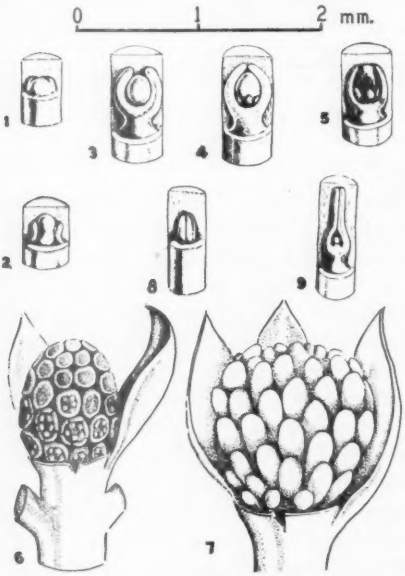
Most desert annuals in California flower in the same month each year (in early spring), irrespective of when germination took place. In years with early winter rains, germination occurs early and the annuals reach considerable size before they start to flower. When the rains come late in winter, the annuals have only a short period of vegetative development and flower while still small. This indicates that they may be long-day plants. Preliminary experiments with about eighty of these annuals showed that many of them actually are long-day plants and do not flower in a daily 8-hour photoperiod but do flower when the 8-hour photoperiod is supplemented with 8 or 16 hours of artificial light. The plants which grew best and flowered first under controlled conditions and artificial light were *Baeria chrysostoma* (minimum of 19 days between germination and anthesis) and *Phacelia parryi* (minimum of 23 days). Both plants are small and therefore can be grown in great numbers in a limited space (one plant per 2 cm.$^2$), making them excellent experimental material.

METHODS.—Seeds of *Baeria chrysostoma* and *Phacelia parryi* were obtained from the Rancho Santa Ana Botanic Garden, through the courtesy of its Director, Mrs. S. Bixby Bryant. The seeds were sown in washed and sterilized silica sand in small glass cylinders, 4 × 8 cm., with open bottoms for drainage. They were covered with sand and after germination were thinned to about five plants per cylinder. The seeds were watered with Hoagland's nutrient solution once every 2 days. By not watering too often and by sterilizing the cylinders and sand together (20 minutes at 2 atmospheres), damping-off of the seedlings could be controlled. Whenever necessary, the plants were sprayed against aphids with an oil emulsion (Extrax). The plants were all grown in artificial light of about 250 f.c., from fluorescent bulbs in rooms with controlled temperature and humidity (10). As a short photoperiod, exposure of 8 hours daily, usually from 8 A.M. to 4 P.M., was employed throughout the investigation. Except where noted, long photoperiod meant continuous exposure to the artificial light; one long photoperiod was exposure from 8 A.M. one day until 4 P.M. the next day.

Under the conditions of these experiments, the development of *Baeria* is as follows:

The seeds germinate within 24 hours after sowing. In another 24 hours the two cotyledons have appeared. These

are linear and almost cylindrical and in the first few days reach a length of 10–14 mm. The growing point can be seen only by pulling the cotyledons apart. All further observations are best carried out




FIGS. 1–9.—Apical growing points of *Baeria chrysostoma* (front leaf pulled off, leaving semicircular scar): Figs. 1–5, successive stages of flower primordia; in all cases, two youngest opposite leaf primordia are separated by flower primordium, which has just initiated involucral bracts in fig. 4 and florets in fig. 5. Figs. 6, 7, later stages; two highest foliage leaves and 2–3 involucral bracts removed to show flower head. Fig. 8, growing point of purely vegetative plant; only two leaf primordia visible; small apical meristem hidden between them. Fig. 9, growing point kept at 28° C. in continuous light; flower primordium initiated but not developing further and overgrown by leaf primordia.

under a preparation microscope with a 20–40× magnification. Practically the whole growing point differentiates into two opposite leaves, with almost no meristematic tissue between. Soon the first pair of foliage leaves starts to elongate (fig. 8), about 4 days after germination. Almost as soon as the growing point between these leaves enlarges, it divides again into two leaf primordia, with very little meristem between. Simultaneously with the leaf growth, the first internode elongates a few millimeters. This process is repeated as long as the plant remains vegetative, and a succession of opposite linear leaves develops. Only on long photoperiod and high intensities is a narrow leaf lamina formed.

As soon as the plant changes to the flowering condition, the originally hardly visible growing point rapidly widens, resulting in a third cellular dome between the two leaf primordia (fig. 1). At this very early stage of flower differentiation, no mistake can be made between this and the vegetative growing point. Further development of the flower head is shown in figures 2–7. There was a 12-day time difference between figure 1 and figure 5 (plants grown in continuous light). The growing point enlarges to a ball-shape, and then first the involucral bracts and later the individual flowers are differentiated in acropetal succession. The number of flowers per head is variable; any number between seven and fifty may be encountered. Differentiation of the individual flower proceeds as follows: first a ring appears, later giving rise to the corolla (figs. 5, 6, upper flower primordia); then the five stamens are differentiated, and simultaneously the meristematic ring splits into five corolla lobes (fig. 6, lower flowers); further differentiation can be observed only by cutting away the rapidly growing corolla (fig. 7).

After flower initiation, but while the plants are still kept under poor growing conditions (high temperature), the flower primordium does not enlarge further and is being overgrown by the youngest leaf primordia (fig. 9).

In the following descriptions the various stages of flowering are referred to by number, for simple calculation of averages for flower formation. The vegetative stage (fig. 8) is designated as 0. The various stages of flower formation have the same number as that of the figure: the growing point of figure 1 is in stage 1, that of figure 2 is in stage 2, etc. Figure 9 is referred to as stage $\frac{1}{2}$. It is possible to judge miscroscopically a few days after long photoperiodic treatment whether initiation has occurred or not; macroscopically it takes 1–2 weeks longer. In the present experiments floral initiation occurred generally after three pairs of leaves were formed, only rarely after the second pair.

All plants grown under the laboratory conditions of low light intensity remain small and depauperate, when compared with plants growing in nature. Whereas in nature the plants generally branch more or less freely (4, fig. 10*a*), in these experiments branching was not so common. Figure 10*b* shows the general appearance of a *Baeria* plant 25 days old grown in continuous light of 300 f.c.

## Results

In preliminary experiments it was found that only when *Baeria* was kept under long photoperiods did flowering occur, irrespective of light intensity. No plants kept daily for 8 hours in normal daylight and 16 hours in darkness showed signs of flowering, either in summer or in winter.

The first experiments were conducted in a room kept continuously at 28° C., and artificial light of about 250 f.c. was used. Both germination and growth were poor under these conditions, and within 40 days all plants died without the formation of flowers, even when kept in continuous light. Microscopic examination showed no floral initiation after 4, 5, 6, or 7 days of continuous light, starting 3 days after germination. The observations were made at intervals of 2 days, starting at 11 days and ending 20 days after the beginning of the treatment. Only occasionally (about 20%) was stage $\frac{1}{2}$ (fig. 9) observed but never any of the stages of flowering of figures 2–7. During this period there was considerable growth of the second pair of leaves (from 2 mm. to 8 mm.).

FIG. 10.—*A*, *Baeria chrysostoma* var. *gracilis* after MUNZ (4); *B*, as grown at 22° C. in continuous light, 25 days after germination.

Later it was attempted to give the plants a cold treatment, by leaving them for 1–7 days in a cool room kept at 22° C. day and night, afterward returning them to 28° C. Stage $\frac{1}{2}$ was reached in only 10% of the plants; all others were purely vegetative. In another experiment the plants were kept only during the 16 dark hours at 10° C. and for 8 hours each day at 28° in artificial light. Subsequent long photoperiodic treatment at 28° with 5, 7, and 9 days of continuous light did not result in flowering.

Table 1 gives the data for an experiment in which the temperature was kept at either 28° or 22° C. during the photoperiod. Only those plants which received continuous photoperiod at 22° flowered irrespective of their pre-treatment. Neither short photoperiods at 22° nor long ones at 28° resulted in flowering. All subsequent experiments were therefore carried out at a constant temperature of 22°, with no further work at the higher temperature.

TABLE 1

RELATION BETWEEN TEMPERATURE AND PHOTOPERIOD AS AFFECTING FLOWERING IN BAERIA. ALL PLANTS (7–8 PER TREATMENT) OBSERVED 23 DAYS AFTER GERMINATION. PLANTS SUBJECTED AS INDICATED TO PRELIMINARY TREATMENT FOR 8 DAYS; SUBSEQUENT TREATMENT FOR ANOTHER 8 DAYS; FINAL UNIFORM TREATMENT FOR LAST 7 DAYS AT 22° C. ON SHORT (8-HOUR) PHOTOPERIOD

| Subsequent treatment 8 days | Preliminary treatment 8 days | 8-hour photoperiod | | 24-hour photoperiod |
|---|---|---|---|---|
| | | 28° C. | 22° C. | 28° C. |
| 8-hour photoperiod | 22° C. | ..... | ..... | Vegetative |
| 24-hour photoperiod | 28° C. | ..... | Vegetative | Vegetative |
| | 22° C. | Flowering | Flowering | Flowering |

The number of long photoperiods required to induce flowering was investigated. The plants were germinated and after 5 days were exposed to long photoperiods for 5, 6, 7, 8, 9, or 10 consecutive days. They were then returned to an 8-hour photoperiod. They were observed microscopically either 6 or 13 days after termination of the treatment. After 6 days, the 5, 6, and 7 long photoperiods had not resulted in visible flower differentiation; but in the 8, 9, and 10 long photoperiods, only two out of thirteen plants remained vegetative, six were in stage 1 and five in stage 2 or 3. After 13 days only one plant had remained vegetative, and all twenty-seven others which received 5–10 long photoperiods were in some stage of flower differentiation; the plants kept on 5 and 6 long photoperiods had not progressed as far as the others.

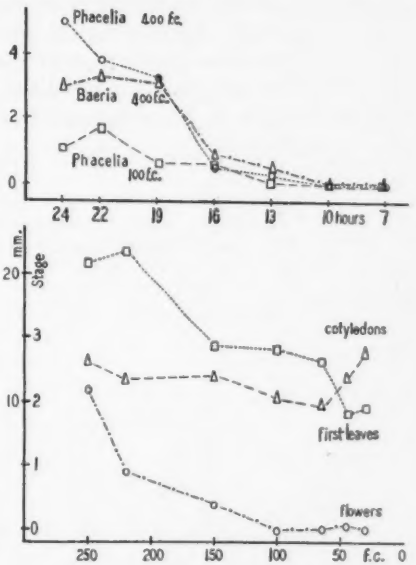
When plants of *Baeria* were exposed to 8 long photoperiods immediately after germination, or 1, 2, 3, or 5 days later, observation 7 days after conclusion of the treatment showed that all plants exposed (starting 5 days after germination) were in an advanced stage of flower differentiation, but in those exposed 0, 1, 2, or 3 days after germination the results were variable: nine plants remained vegetative, nine were in stage 1, and four had progressed to stages 2–3. Apparently the plants become responsive to the long photoperiods very soon after germination. In another experiment, the long photoperiodic treatments were started 4, 7, 10, 13, or 16 days after germination. Whereas after 4 days, 11% of the plants remained vegetative, 9% did not flower when 7–16 days elapsed between germination and exposure to long photoperiods. Therefore the plants have reached full sensitivity to long photoperiods 4 days after germination. In this same experiment it was found that 0, 1, and 2 long photoperiods were completely ineffective in inducing flowering; 8 and 10 long photoperiods gave 100% flowering, 4 long ones gave 38% flowering (stage 1), and 6 long photoperiods gave 79% flowering (stages 1–3). These experiments show that 4 long photoperiods are not quite enough to initiate flowers in 50% of all plants, but 8 such periods are fully effective, whereas further growth of the flower primordia is facilitated by extended exposures to long photoperiods.

To determine the daily photoperiod re-

quired for flower initiation, a long cabinet was constructed which had a sliding cover, by which the plants growing inside the seven compartments of the cabinet could be shut off in succession from the light of fluorescent tubes mounted above the cover. A motor activated by a timeclock pulled this cover back, one compartment at a time. By regulating the timeclock, successive compartments could be exposed to light for successively shorter periods. At a set time the sliding cover was pulled back, so that the plants in any one compartment were exposed to the same photoperiod each day.

In figure 11 the results of one such experiment are shown. Plants of *Baeria* and *Phacelia* were germinated in daylight in a greenhouse. Thirteen days later the plants of *Baeria* had two fully developed first leaves. These plants were then placed in the cabinet at 20°C. constant temperature. Ten days later the plants were observed microscopically and their average flower development expressed in a number corresponding to their stage (ordinate in fig. 11). Whereas there was no trace of flower initiation on the 7- and 10-hour photoperiods, on a 13-hour photoperiod three out of ten plants had reached stage 1, the rest remaining vegetative; on a 16-hour photoperiod only three out of ten plants were still vegetative, whereas on the 19-, 22-, and 24-hour photoperiods flower formation had progressed to stages 2–4 and no vegetative plants were found. Comparable plants, exposed in the greenhouse to the natural 10-hour photoperiod of middle December, were all vegetative, except four out of twenty, which were in stage 1–2. Apparently 15 hours of daylight is the critical photoperiod for *Baeria* at the low light intensities employed. The critical photoperiod for *Phacelia* also is 15 hours (fig. 11), and at lower intensity there is little further development of the flower primordia.

In other experiments, the same 15-hour critical photoperiod was found; it is clear, however, that this critical photoperiod is not as sharply defined as in *Xanthium* (3), but more like that in soybeans (1).




FIGS. 11, 12.—Fig. 11 (above), relation between length of photoperiod (abscissa, in hours) and flowering of *Baeria* (triangles) and *Phacelia* (circles, 400 f.c.,; squares, 100 f.c.). Flowering stages plotted on ordinate, o being vegetative. Fig. 12 (below), length of cotyledons (triangles) and first leaves (squares) of *Baeria* plants grown at 22° C. and continuous light of different intensity (abscissa, in foot candles). Also flowering plotted (circles) in terms of stage reached (ordinate, o = vegetative).

The lowest light intensity which could still induce flower formation was determined. The plants were either grown continuously in different light intensities or kept for 8 hours each day in 250 f.c., being subjected to different intensities for the other 16 hours (table 2). In continuous light an intensity of more than 200

f.c. is required for formation of fully developed flower buds. At lower intensities only a few flowers develop, or none are initiated. At intensities below 150 f.c. vegetative development was very meager.

Figure 12 shows the results of an experiment in which plants of *Baeria* were kept 5–13 days after germination at 300 f.c. for 8 hours each day, and for the remaining 16 hours at different intensities. Then they were returned to an 8-hour photoperiod of 300 f.c. only. Measurements were made 19 and 25 days after germination (6 and 12 days after the end of treatment). Length of the cotyledon was not materially affected by the light. On the other hand, length of the first pair of leaves was much greater at the two highest intensities. Flowering was induced only by exposure to light of more than 100 f.c.; beyond that there was a marked effect of intensity on the further development of the flower primordium.

The effect of different colors of light (all of 250 f.c. intensity, obtained by passing light of a 1000-watt incandescent light through Corning glass filters) when used in addition to a daily 8-hour exposure to 1500 f.c. is shown in table 3. As expected, the controls were completely vegetative but had fairly good leaf development. In green light the plants were also vegetative, and their leaf growth was about the same as the controls kept in darkness. Red was most effective for flowering, then yellow, and blue was least effective. In another experiment the effects of blue, yellow, and red were approximately the same for flowering, but the controls and plants in green light were vegetative.

In an earlier experiment lower intensities (below 100 f.c.) of colored light were used as additional light to an 8-hour photoperiod in white light. As was to be expected, none of the colors induced flowering. At least 100 f.c. of additional light are needed to induce flowering (table 2).

An experiment was carried out to determine the effectiveness of the different pairs of leaves in inducing flowering. Plants of *Baeria* were germinated, and from 5 days after germination on, kept in continuous light. When the cotyledons and two pairs of leaves were left, all

TABLE 2

FIVE DAYS AFTER GERMINATION, BAERIA PLANTS WERE PLACED CONTINUOUSLY IN DIFFERENT LIGHT INTENSITIES. OBSERVATIONS ON DEVELOPMENT AND FLOWERING WERE MADE AFTER TWO TIME INTERVALS

| LIGHT INTENSITY (F.C.) | 17 DAYS AFTER GERMINATION | | | | | STAGE OF FLOWERS 29 DAYS AFTER GERMINATION |
|---|---|---|---|---|---|---|
| | No. of leaves | Length (mm.) | | | Stage of flowers | |
| | | Cotyledons | First two leaves | Second two leaves | | |
| 250 | 4 | 8 | 13 | 11 | 5, 5, 5, 5, 4, 2, 1 | 7, 7, 7 |
| 206 | 6 | 12 | 19 | 12 | 6, 5, 4, 3, 3 | 7, 7, 7 |
| 162 | 4 | 12 | 20 | 5 | 5, 5, 1, 1 | 1, 1, 1, 0 |
| 118 | 0 | 16 | < 1 | < 1 | All 0 | 5, 1, 1, 1, 0 |
| 74 | 0 | 15 | < 1 | ..... | All 0 | 1, 1, 0, 0 |
| 30 | 0 | 11 | < 1 | ..... | All 0 | 0, 0, 0 |

plants showed floral initiation 17 days after germination. When either the cotyledons or the first or second pairs of leaves were cut off but the others left (cotyledons and one pair of leaves or only two pairs of leaves), all plants showed flower primordia at 17 days. When only the cotyledons or the first or second pair of leaves were left on the plants, some had flowers initiated but others had not, and there seemed to be no difference between the groups. Apparently the cotyledons and leaves contribute equally to the change from vegetative to flowering in *Baeria*.

From the previous experiments it had become clear that plants of *Baeria* kept on short photoperiod did not flower, but that they could be used to test the effectiveness of various additional treatments in flower initiation. Since it was known that caulocaline and sugars could be absorbed by leaves submerged in solution (11), and since the linear leaves can easily be inserted in glass capillaries of solutions, experiments were run to study the effectiveness of pure substances and extracts on flower initiation. Plants were grown on 8-hour photoperiods, and as soon as either the cotyledons or first leaves were well developed they were inserted in capillaries thrust in the sand of the container. Each day the solution in the capillaries was renewed. None of the treatments resulted in initiation of flowers. The experiments are being continued. Substances tried were 3% and 6% sucrose, thiamin, pyridoxine, nicotinic acid, pantothenic acid, para-aminobenzoic acid, and biotin (all in 1 p.p.m.), and tomato and palm leaf extracts.

TABLE 3

LEAF DEVELOPMENT AND FLOWERING OF BAERIA PLANTS EXPOSED DAILY TO 8 HOURS OF WHITE LIGHT AND 16 HOURS OF COLORED LIGHT OF 250 F.C. (COLORS OBTAINED WITH CORNING GLASS FILTERS). TREATMENT WITH COLORED LIGHT FROM 5–13 DAYS AFTER GERMINATION; OBSERVATION AFTER 19 DAYS

| Color of additional light | Approximate wave lengths | No. of leaves | Length (mm.) | | | Stage of flowering |
|---|---|---|---|---|---|---|
| | | | Cotyledons | First pair of leaves | Second pair of leaves | |
| Darkness | | 4 | 9.1 | 11.6 | 1.7 | 0.0 |
| Red | 5700–8000 | 6 | 8.3 | 20.6 | 17.6 | 3.2 |
| Yellow | 5000–8000 | 4 | 10.5 | 14.8 | 2.3 | 2.0 |
| Green | 4700–5800 | 3 | 9.0 | 12.1 | 1.0 | 0.0 |
| Blue | 3500–5300 | 4.6 | 9.6 | 17.3 | 13.7 | 1.0 |

## Discussion

*Baeria* is a convenient plant for photoperiodic investigations, owing to its quantitative response, which makes it possible to express effects of light in terms of numerals. It can also be grown in low light intensities and responds quickly to treatment. The plants respond to a long photoperiodic treatment 5 days after planting, since two pairs of leaves, or the cotyledons and one pair of leaves, suffice for photoperiodic perception. As soon as cotyledons and the first pair of leaves are fully developed, the plant becomes sensitive to long photoperiods. In the case of millet (2) the plants become sensitive after 1 week, in soybeans (1) after 3 weeks.

Among photoperiodically sensitive plants there is a distinction between those in which there is a very narrow range in photoperiods giving either flowering or no flowering (as *Xanthium*, 3), or where there is a gradual change to flowering over a rather wide range of photoperiods (as soybeans, 1). *Baeria* belongs to this second group.

There also are differences in the intensity of supplementary light required to induce photoperiodic responses. In general, this intensity can be very low, mostly 10 f.c. or less (in aster even 0.3 f.c. is fully effective, 12). In *Baeria*, on the other hand, higher intensities are required of the same magnitude as necessary for photosynthesis. This may be the reason why the spectral sensitivity of *Baeria* differs so much from that of most other plants. Aster, stocks, soybeans, spinach, and many others (13) need red or yellow supplementary light to initiate flowers; blue light is completely ineffective. In *Baeria* only green light is ineffective, but both the long and the short wave lengths induce flowering. It would seem that photosynthesis beyond a 10-hour photoperiod required for vegetative development induces flower initiation, for the light intensity during the 10-hour short photoperiod can be varied within a very wide range (200–1500 f.c. or more in daylight) without resulting in flowering, whereas the supplementary light is effective whether during short photoperiods the plants were subjected to high or to low light intensity. For short-day plants photosynthesis is probably the controlling factor during the short photoperiod (7).

In *Baeria*, long photoperiod is effective only in inducing flowers when the plants are kept below 25° C. Such a relation between photoperiodic behavior and temperature has been known to exist in many plants (9, 8). According to ROBERTS and STRUCKMEYER, alfalfa, pea, nasturtium, primrose, and red clover are all examples of long-day plants which do not flower when kept on long warm days. But *Baeria* differs very much from such long-day plants as sugar beet and *Rudbeckia*, in which low or high temperatures may substitute for long photoperiods (6, 5).

HAMNER and BONNER (3) tried the application of many extracts or pure substances to *Xanthium* kept on long photoperiods, but in no case did they induce flowering. The present experiments with applied substances were equally unsuccessful.

## Summary

1. *Baeria chrysostoma* is a long-day plant, provided it is grown below 25° C. It will grow at light intensities of above 150 f.c., obtained with fluorescent tubes, and responds rapidly to long photoperiods.

2. At least 5 long photoperiods are required for flower initiation, and a 15-hour photoperiod is effective. Plants become sensitive to long photoperiods 5 days after germination; and a few days after the long photoperiodic treatment, microscopic observation shows flower initiation.

3. The intensity of the supplementary light required after an 8-hour photoperiod for flower initiation is above 100 f.c. Both the short and the long wave lengths are effective, but green light is not. The cotyledons and leaves are equally effective in perception of the light effect.

4. No extracts or pure substances induced flowering in plants kept on short photoperiods.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. BOT. GAZ. 100:374–387. 1939.
2. CAJLACHJAN, M. C., The age of plants and the photoperiodic reaction. Compt. Rend. Acad. Sci. U.R.S.S. 6:306–314. 1933.
3. HAMNER, K. C., and BONNER, JAMES, Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1939.
4. MUNZ, A. P., A manual of Southern California botany. Claremont, California. 1935.
5. MURNEEK, A. E., Length of day and temperature effects in *Rudbeckia*. BOT. GAZ. 102:269–279. 1941.
6. OWEN, F. V., CARSNER, E., and STOUT, M., Photothermal induction in sugar beets. Jour. Agr. Res. 61:101–124. 1940.
7. PARKER, M. W., and BORTHWICK, H. A., Floral initiation in Biloxi soybeans as influenced by photosynthetic activity during the induction period. BOT. GAZ. 102:256–268. 1941.
8. ROBERTS, R. H., and STRUCKMEYER, B. E., Further studies of the effects of temperature and other environmental factors upon the photoperiodic responses of plants. Jour. Agr. Res. 59:699–710. 1939.
9. THOMPSON, H. C., Temperature as a factor affecting flowering of plants. Proc. Amer. Soc. Hort. Sci. 30:440–446. 1934.
10. WENT, F. W., Plant growth under controlled conditions. I. The air-conditioned greenhouses at the California Institute of Technology. Amer. Jour. Bot. 30:157–163. 1943.
11. WENT, F. W., and BONNER, D. M., Growth factors controlling tomato stem growth in darkness. Arch. Biochem. 1:439–452. 1943.
12. WITHROW, R. B., and BENEDICT, H. M., Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daylight period. Plant Physiol. 11:225–249. 1936.
13. WITHROW, R. B., and WITHROW, A. P., Effect of various wavebands of supplementary radiation on the photoperiodic response of certain plants. Plant Physiol. 15:609–624. 1940.

# CYTOLOGY AND MICROCHEMISTRY OF NUCLEI IN DEVELOPING SEED OF ECHINOCYSTIS MACROCARPA

FLORA MURRAY SCOTT

## Introduction

Nuclei of relatively enormous size have been described in the differentiating vessels of *Ricinus communis* and of *Echinocystis macrocarpa* (12, 13). In the developing seeds of *Echinocystis*, even larger nuclei appear and are visible to the naked eye.

In reference to rapidly segmenting animal eggs, PAINTER asks, "How is it possible for the chromosomes in rapidly segmenting eggs to synthesize so quickly the new material needed in the reduplication process prior to each cell division?" It is seen that in *Drosophila* the nurse-cell nuclei increase about 512 times in volume. Cells and nuclei are later absorbed by the developing egg. From further evidence such as this PAINTER concludes that "the rapid building of the cleavage chromosomes is possible in the segmenting egg because the synthesis is more in the nature of a reassembling of already existing materials such as nucleotids, etc. under the guidance of active chromosomes rather than an actual synthesis of building blocks from relatively simple substances" (10).

In botanical terms this question may be restated as follows: How is it possible for the chromosomes to synthesize the materials necessary for the innumerable mitoses which result in the dense small-celled tissue of the rapidly developing embryo cotyledons? In the plant, nucellus and endosperm replace the nurse cells and presumably function

as the immediate source of the building materials necessary for nuclear and cytoplasmic protein synthesis.

The present paper serves as an introduction to the cytology and microchemistry of the giant nuclei present in the nucellus and endosperm of the developing seed.

MATERIAL AND METHODS.—In southern California, *Echinocystis macrocarpa* flowers more or less continuously from January until about the end of April. Fresh material in all stages of growth is therefore available throughout this period. The inferior 4–6 locular ovary measures 0.3–0.5 cm. in length. Locules may contain 4–6 seeds, some of which may fail to develop. The mature spiny fruit measures 7–10 cm. in length.

Undoubtedly the most significant recent tool in cytological investigation is the ultraviolet technique of CASPERSSON (1). In the wartime absence of essential equipment, microchemical tests must necessarily suffice in a survey of the distribution of chromatin and other ergastic substances. The following methods or reagents were used: (*a*) Feulgen's reaction–nucleic acid (**6, 8, 9**); (*b*) ninhydrin, triketohydrindene hydrate–amino acids (**7**); (*c*) Millon's reagent–tyrosin; (*d*) trypsin, with or without the addition of lanthanum chloride–protein digestion (**1**); (*e*) Fehling's solution–reducing substances (**15**); (*f*) Sudan III–fats; (*g*) iodine potassium iodide; and (*h*) ferric chloride–starch and tannin, respectively. The amount of free water present in the nucleus was determined as before by dehydration in absolute alcohol (**12, 13**).

In preliminary observations on pH, the range indicator method was used (**14**). In addition to the standard series of dyes, thionin (Lauth's violet) also proved of interest (**3**). Permanent preparations were stained with haematoxylin, with or without such counterstains as erythrosin and orange G. So far no fixative has been found which prevents considerable shrinkage.

## Observations

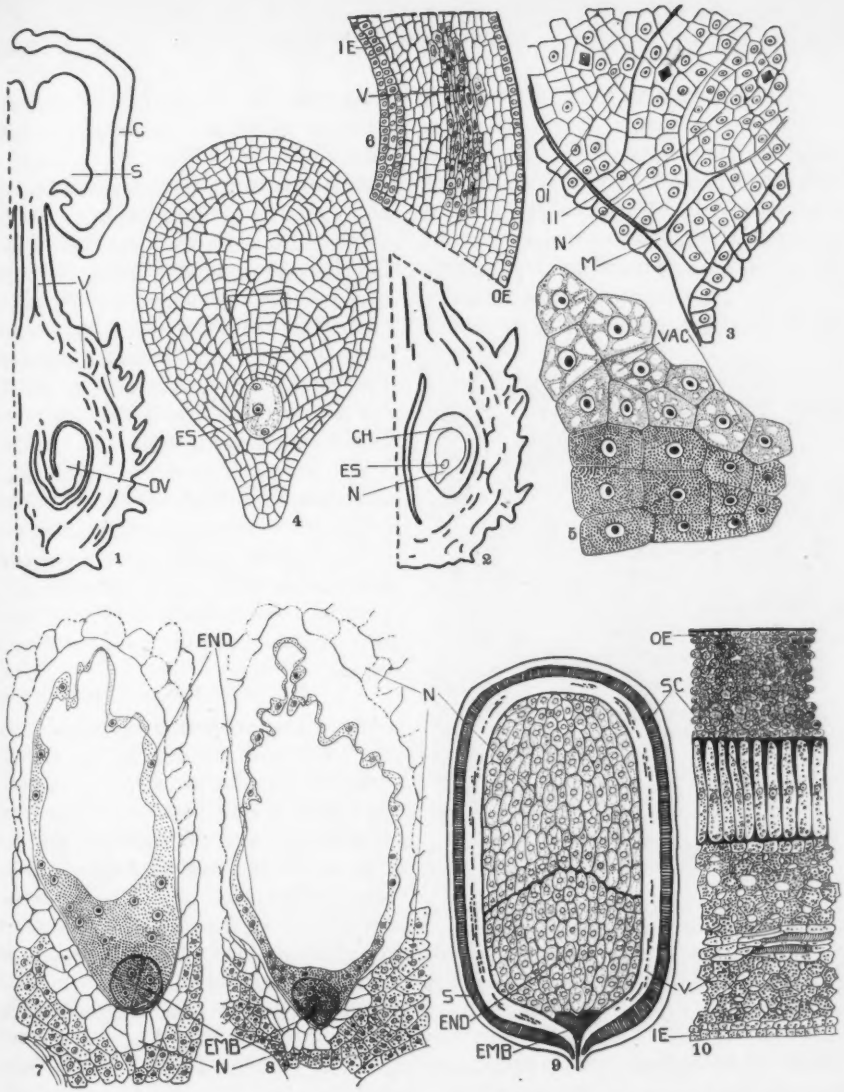
### MORPHOLOGY OF SEED DEVELOPMENT

Differentiation of the megagametophyte, fertilization, and subsequent growth of the seed follow the general pattern described by KIRKWOOD (**4**) and KRATZER (**5**) for the Cucurbitaceae as a whole. A brief summary will therefore suffice at this time.

The megagametophyte when mature is deeply imbedded in the nucellus of the anatropous ovule. Two integuments are present—the inner slight, only three cells thick; the outer complex and many-layered. In the latter a differentiating vascular network is already apparent. The main axis of this reticulum encircles the seed and is continuous with the funicular vascular strand. In the mature seed it underlies the definitive dark ridge of the seed coat. The nucellus extends into the micropyle as a nucellar neck. The ovule at this stage measures 0.5 mm. in length (figs. 1–6).

After fertilization, growth of seed and fruit is rapid, but the embryo remains practically invisible to the naked eye until the seed is more than half-grown. The endosperm is at first coenocytic as usual. Thereafter cell formation begins in the embryonic zone, while the distal endosperm penetrates the nucellus as a noncellular coecum (figs. 7–11).

Mature seeds measure $\pm$ 2 cm. in length. When the seeds first reach maximum volume, the seed coat is white, soft, and unlignified, but is highly differentiated as to outer integument. Starch is abundant in all except the palisade cells.




FIGS. 1–10.—Diagrammatic drawings: Fig. 1, unopened flower, longisection showing attachment and vascular supply of one ovule. Fig. 2, at another level; position of megagametophyte evident. Fig. 3, same; inner and outer integuments surround micropyle and nucellar neck; mitosis throughout. Fig. 4, same, nucellus and megagametophyte; cells of nucellus immediately adjacent to latter are densely filled with protoplasm, while remainder are already vacuolated. Fig. 5, vacuolating and nonvacuolated cells of nucellus. Fig. 6, testa, longisection; developing vascular strands evident, otherwise seed-coat tissues are relatively undifferentiated; multinucleolate nuclei appear in vascular tissue. Fig. 7, seed, longisection (0.5 mm.); embryo in 8-cell stage with surrounding endosperm. Fig. 8, seed, longisection (2.3 mm.); multicellular embryo. Fig. 9, diagrammatic surface view of seed after partial removal of testa (2 cm.); endosperm, now cellular, occupies nearly half seed cavity; dark line indicates limit of nucellus identifiable on dissection; testa shows micropyle, differentiating sclerenchyma, and vascular tissue. Fig. 10, testa, longisection; sclerenchyma vascular tissue, starch-filled ground cells and intercellular spaces evident. *c*, corolla; *ch*, chalaza; *emb*, embryo; *en*, endosperm; *es*, megagametophyte; *ie*, inner epidermis of testa; *ii*, inner integument of ovule; *m*, micropyle; *n*, nucellus; *nc*, nucleus; *ncl*, nucleolus; *oe*, outer epidermis of testa; *oi*, outer integument of ovule; *ov*, ovule; *s*, stigma; *sc*, sclerenchyma of testa (differentiating); *v*, vascular and provascular tissues; *vac*, vacuoles.

The seed cavity is filled with a firm gelatinous transparent ellipsoidal mass, nucellus and endosperm. The surface cells are small, but the inner cells and nuclei are visible to the naked eye. The endosperm gradually encroaches upon and absorbs the nucellus. Morphologically these tissues are indistinguishable in surface view, but when separation is

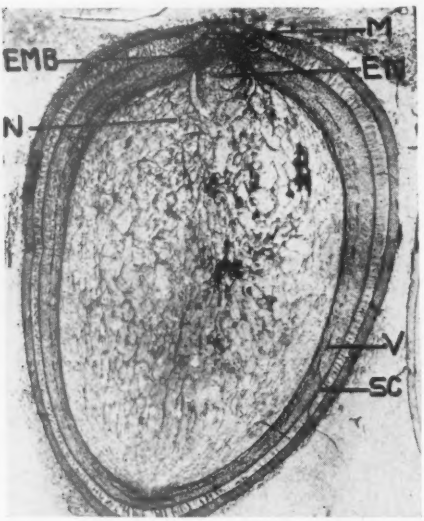



FIG. 11.—Seed, diagrammatic longisection (1.3 cm. after imbedding); embryo, cellular endosperm, region of coecum penetration indicated by partial breakdown of surrounding nucellus, and intact cellular nucellus occupy seed cavity.

effected by careful probing the smaller cells of the endosperm surface layer are apparent (figs. 9–11). All cells are highly turgid, and when punctured the contents spurt out forcibly in all directions. When the cells are so punctured the nuclei are set free and float in the cytoplasmic fluid. No mounting fluid is required, and this material is therefore excellent for nuclear investigation.

Hardening of the seed coat begins with the lignification, near the micropyle, of the palisade layer as it develops into sclerenchyma tissue. Within the seed cavity, while the nucellus is being gradually resorbed by the endosperm, both are in turn being consumed by the now rapidly expanding embryo. During this resorption the cells lose their turgor, the cell walls gradually break down, the nuclei shrink in volume, and the whole tissue disintegrates in a semifluid, somewhat viscous mass and finally disappears. The embryo cotyledons occupy the entire seed cavity. Tannin-like substances darken the ripe seed coat.

The comparative sizes of fruit, seed, and embryo, and also of cell and nuclear size in endosperm and nucellus, are seen in the typical measurements given in table 1. The visibility of cells and nuclei is readily understood on examination of their dimensions in the full-grown seeds.

## NUCLEAR MORPHOLOGY

When the megagametophyte is mature, the cells of the nucellus are already varied in size and in extent of vacuolation. Surface cells, the cells of the neck and those immediately around the embryo, are smaller and contain relatively dense protoplasm. The larger central cells are vacuolated. Cell division is active throughout and all stages of mitosis are seen (figs. 4, 5).

Cell division is practically complete about the time of fertilization, and increase in nucellar volume results from cell expansion. This is clear from the cell count along the major and minor axes of the elliptical nucellus as seen in longitudinal section (fig. 4). Thus at this early stage the number of cells along the longitudinal and transverse axes is $\pm$ 19 and $\pm$ 14, respectively. In a typical seed practically full-grown, the cells across the latter, the minor axis, number $\pm$ 14. In the meantime, in the seed cavity endosperm has replaced approxi-

mately one-half the nucellus. Along the remaining major radius of the nucellus the cells now number ± 9 (fig. 9). The role of cell expansion is thus clearly indicated. In the endosperm cell division is completed soon after fertilization, and since the endosperm cells parallel the nucellar in volume increase, the nutritive tissue—during development—superficially appears homogeneous.

In the young nucellus about the time of fertilization, the nuclei of all cells are relatively uniform, and it is possible to describe a "typical" nucleus. Such a body measures 7–10 μ in diameter. Except during actual division, the chromatin appears in the interkinetic "resting" condition. One nucleolus is present as a rule, but two or occasionally three may be observed (fig. 5). In specialized cells only, such as the differentiating vascular elements of the outer integument, nucleoli are more frequent and range 4–7 in number (fig. 6).

In older seeds, endosperm and nucellar nuclei so far remain morphologically and microchemically indistinguishable, but it is no longer possible to talk of a typical nucleus. While superficially the chromatin network appears similar throughout, the nucleolar condition is chaotically variable. The chromatin consists of strands which vary in thickness from 0.5 to 3 μ. The latter may be more or less clearly observed in all types of preparations: in living nuclei before or after vital staining, in transmitted light or in dark field, in preparations stained with acetocarmine or with microchemical reagents such as Feulgen or ninhydrin, and in permanent haematoxylin-stained mounts. The orientation of the major strands and of the larger nucleoli is interdependent. The thicker chromatin threads invariably appear to radiate from the latter. The lesser nucleoli also occur at chromatin-strand intersections (figs. 12–16).

The finer chromatin strands present the usual "beaded" appearance. The thicker vary erratically in width throughout their length and at intervals may be clearly resolved into a number (generally 3–5) of finer component beaded threads. The theoretical concept of the multiple structure of the salivary chromosomes of *Drosophila* is thereby suggested. In this phase of nuclear development, however, structural details in the reticulum cannot be definitely resolved by ordinary optical means. It appears probable that increase in chromatin depends on the multiplication of the chromonemata and occurs during cell and nuclear expansion. The conditions which favor volume increase are therefore identical with, or accompany, those which inhibit the completion of mitosis.

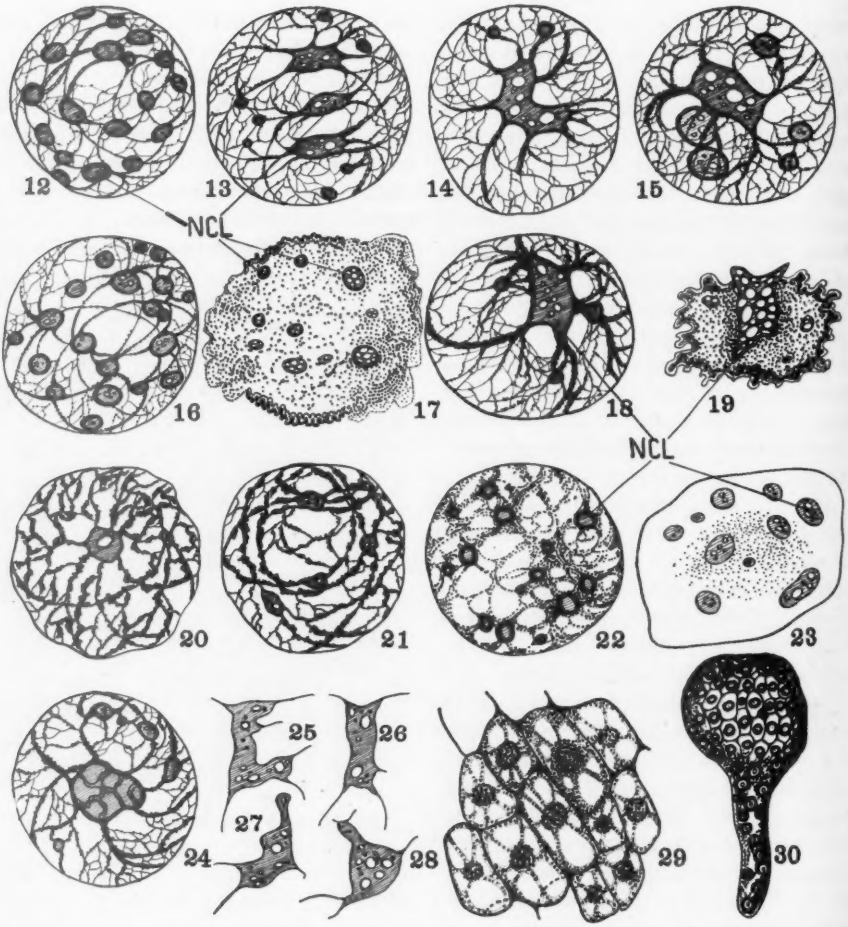
TABLE 1

MEASUREMENTS OF FRUITS OF VARYING SIZE AND TYPICAL INCLOSED SEED EMBRYOS, CELLS, AND NUCLEI

| Fruit length (cm.) | Seed length (cm.) | Cotyledon length (cm.) | Cell size* (μ) | Diameter of largest nuclei (μ) |
|---|---|---|---|---|
| 1.4 | 0.2 | Microscopic | 76 | 15 |
| 2 | 0.6 | Microscopic | 256 | 40 |
| 3.2 | 1 | Microscopic | 350 | 64 |
| 4.2 | 1.4 | 0.05 | 660 | ..... |
| 5.8 | 1.7 | ..... | ..... | 104 |
| 7.5 | 2 | 0.5 | 800 | 114 |
| 8 | 1.9 | 0.5 | 800 | 123 |
| 8 | 2.0 | ..... | 2800 | 200 |

* Majority are ellipsoidal; mean of major or minor axes recorded here.

The nucleoli vary in size, in consistency, and in outline. Diameters vary from 0.2 to 40 μ, and may therefore be only roughly graded as large, inter-




FIGS. 12–30.—Figs. 12–15, nuclei (diameters 152, 104, 95, 96 μ); nucleoli varied in size and number. Figs. 16, 18, nuclei (190 and 120 μ) before digestion. Figs. 17, 19, same during digestion in trypsin. Figs. 20, 21, after treatment with trypsin and lanthanum chloride and subsequent staining in Feulgen; nuclear structure persists. Fig. 22, nucleus (diameter 95 μ); after treatment with Millon. Fig. 23, nucleus (major and minor axes, 152 and 95 μ) during treatment with Fehling's. Fig. 24, nucleus (diameter 57 μ); nucleolus fragmented. Figs. 25–28, nucleoli of varying outlines. Fig. 29, nucellar cells after treatment with Fehling's; density of cuprous-oxide precipitate indicated by black granules. Fig. 30, differentiating endosperm dissected out; 15 free nuclei in noncellular coecum (4800 μ). Semidiagrammatic drawings of nuclei (figs. 12–28) based on camera-lucida outlines.

mediate, and small. Values such as 30 μ, 15 μ, and 5 μ may be arbitrarily assigned to these terms in order to clarify general morphological description. Nucleolar composition is heterogeneous. Vacuoles of undetermined content and of all sizes are scattered throughout. In smaller nucleoli the conventional spherical or elliptical outline is generally maintained, but in the larger ones fantastic forms occur. Such outlines presumably result from internal changes, accompanied by alterations in surface tension. After a survey of some 250 living nuclei, and later intensive study of permanent preparations, only a few generalizations appear valid in relation to nucleolar number, outline, and distribution:

1. In nuclei 10–20 μ or less in diameter, only one nucleolus is present as a rule, but occasionally two may appear (fig. 5).

2. When nuclear diameter ranges 25–40 μ, the nucleoli may number 1–4. Exceptions occur, however, and 5–7 nucleoli are sometimes observed.

3. Conditions in the larger nuclei, 40–200 μ, are chaotic. No correlation is at present evident between nuclear volume and nucleolar number. Various patterns are observed: (*a*) All nucleoli are comparatively small, and their number appears indefinite (fig. 12). They constitute the entire nucleolar material. (*b*) Nucleoli of intermediate size and relatively limited number (fig. 13) are dominant. They are almost invariably accompanied by one or several smaller units. (*c*) The number of large nucleoli is necessarily spatially limited. One to three may occur, but, as before, one or more of the smaller elements is usually present. The outline may be conventional, spherical, elliptical, or entirely erratic (figs. 14, 15, 25–28). (*d*) Some of the largest nucleoli appear fragmented. The nucleolar membrane remains intact, but the body is subdivided into irregular segments, as if ready to split apart. The significance of this condition is not clear. Present evidence suggests that the nuclei increase in number gradually, not suddenly, as would be the result of sudden fragmentation (fig. 24).

Since nucleolar number is in general considered an index of chromosome duplication, the conclusion is inevitable that, in the largest multinucleolate nuclei, actual polyploidy lies beyond the range of microscopic definition by means of visible light only. Ultraviolet technique may contribute to the detailed analysis of this complexity of structure.

### Nuclear microchemistry

The question of the source of nucleoproteins for the embryo in its phase of rapid growth has already been stated. The full answer will necessarily depend on accurate quantitative analysis by chemical methods, together with ultraviolet techniques. Routine microchemical tests, however, are of use in a general survey.

Feulgen's reagent.—Mild hydrolysis of nucleoprotein followed by the Schiff reaction of the resulting aldehydes produces more or less intense purple coloration throughout the cell. The chromatin network is deeply stained, and the color of the nucleoli is almost equally intense. The karyolymph is lighter in color. Beyond the limits of the nucleus, cytoplasm and cytoplasmic granules, grading down to the limits of visibility, are also distinctly stained. As already demonstrated by Caspersson and Schultz (2), nucleic acid is present in structures other than chromatin.

During nucellar and endosperm resorption, as the embryo develops these disintegrating tissues stain even more

heavily. Remains of shrunken nuclei may still be recognized. Granules are abundant in nuclei and in cytoplasm. Intensity of color depends in part on the concentration of material resulting from loss of water.

During the period of active growth of the embryo, the nutritive tissues, nucellus and endosperm, thus furnish a volume of available nucleoproteins. To what degree these building materials are broken down prior to absorption by the embryonic tissues remains to be determined. Detailed quantitative analysis would yield useful information regarding the amount of material used in embryonic growth compared with the supply immediately available in endosperm and nucellus.

NINHYDRIN.—All naturally occurring amino acids give an intense blue coloration on addition of ninhydrin (**7**), so that this reaction is in no wise specific for any particular amino acid. As expected, at all stages of growth the chromatin, nucleoli, and karyolymph of the nucleus —and also the abundant granules and matrix of the cytoplasm—become, on mild warming (50°–60° C.), more or less deeply stained. Ninhydrin functions almost as well as any chromatin stain in resolving details of the reticulum.

MILLON'S REAGENT.—The presence of the tyrosin complex was noted in the tuber of *Echinocystis* (**13**). Treatment of the developing seeds with Millon's reagent produces an equally positive reaction. Nucellus, endosperm, and embryo are immediately stained more or less deeply reddish brown. Tissue shrinkage occurs and the darkening color completely masks details of structure in the cell as a whole. The free nuclei, however, which are present in any preparation, are readily observed. Loss of nuclear volume is evident on entrance of the reagent, and the characteristic reddish color appears almost immediately. The nucleoli, if irregular in outline, round off and become spherical. In all, the vacuoles merge in the center, and the surrounding reddened crater-like rim of nucleolar matrix remains refringent (fig. 23). Chromatin becomes granular and stains more heavily than karyolymph. The occurrence of this amino acid in nuclei is not unusual.

FEHLING'S SOLUTION.—Reducing sugars are commonly distributed in nutritive as in other tissues. In nucellus and endosperm the reaction to Fehling's solution is readily seen microscopically. On careful and continued addition of boiling KOH (**15**), the cytoplasm and nuclei rapidly change from blue to mustard yellow, to orange, and eventually to deep red-brown. In the cytoplasm, as the color deepens, cuprous oxide precipitate appears around the periphery of the cell, along the radiating cytoplasmic strands, on the surfaces of the vacuoles, and—most conspicuously—as a corona masking the nucleus (fig. 29). In the nucleus itself color change parallels that in the cytoplasm. Details of chromatin outline are soon lost, but nucleoli remain distinct and are equally stained (fig. 23). The nucleus continues to darken, and further observation is effectively blocked by the intensity of the internal precipitate and by the cytoplasmic corona already noted.

This reaction denotes reducing substances within the nucleus itself. Whether these exist free within the karyolymph, or result from hydrolysis of nuclear components during the reaction, is undetermined. Absorption of water might be attributed entirely to adsorption by nucleoproteins, or it may be linked with osmotic substances. In analyses of sperm and similar nuclei so far available,

monosaccharides and similar substances do not appear. Detailed analyses of hypertrophied nuclei of high water content, such as the present, are lacking, however. The identity of these Fehling's reducing substances therefore remains to be determined.

TRYPSIN.—Microchemical dissection by means of enzymes affords further evidence regarding nucleic-acid distribution (2). In qualitative experiments, digestion by powdered trypsin was followed by the Feulgen test. The process of digestion may be observed microscopically and is complete in 15–20 minutes or less. The nucleus shrinks markedly in volume within 3–5 minutes, but details of nucleoli and nuclear network are not thereby obscured. The nuclear membrane appears slightly thicker and thereafter becomes more or less finely puckered. The nucleoli also shrink and very soon increase in vacuolation, but the spongy matrix which results remains visible and highly refringent (figs. 16–19). The nuclear membrane now thins out along one or more sectors and eventually breaks. In the meantime the entire nuclear material has become finely granular. The granules, $\pm$ 1 $\mu$ and grading down to the limits of visibility, are in active Brownian movement and escape on rupture of the nuclear membrane. The enzyme trypsin, by digestion of protein, thus disrupts completely the continuity of the chromatin mesh. The slightest pressure on the coverglass is sufficient to disperse the particles and to remove completely all traces of nuclear outline.

This disintegration may be effectively blocked by addition of lanthanum chloride (figs. 20, 21). Nucleic acid, when freed from the nucleoprotein complex during trypsin digestion, immediately reacts with lanthanum ion and precipitates. The continuity of the chromatin complex remains unimpaired. The contrast between fragmented and intact nuclei is excellently seen on careful addition of Feulgen's reagent.

WATER CONTENT.—To determine the volume of removable water present, the technique of dehydration in absolute alcohol is repeated here (12). The results are given in table 2. As in other large nuclei examined, this volume ranges 60–80%. The actual increase in nuclear structural materials, chromatin, etc., is estimated by comparison of the volumes of a fully contracted giant nucleus and a typical meristem nucleus. In one case (original diameter 104 $\mu$) the volume after dehydration is 117,424 cu. $\mu$, while the volume of a typical meristem nucleus is $\pm$ 33 $\mu^3$. Growth in structural material is therefore approximately 3558-fold. The significance of this increase in nucleoproteins in relation to embryonic growth and food supply has already been mentioned.

TABLE 2

PERCENTAGE VOLUME OF WATER IN NUCLEI INDICATED ON CONTRACTION IN ABSOLUTE ALCOHOL

| INITIAL DIAMETER ($\mu$) | VOLUME (%) | | |
|---|---|---|---|
| | Initial ($\mu^3$) | After contraction ($\mu^3$) | Loss |
| 43 | 43,601 | 14,801 | 63 |
| 57 | 96,762 | 20,900 | 78 |
| 96 | 462,274 | 12,816 | 72 |
| 108 | 658,199 | 62,980 | 81 |

pH.—Protein synthesis is conditioned by pH, in particular by the isoelectric point of the dominant protein, among other factors (11). In meristematic tissues, including the cambium, mitosis alternates regularly with nucleoprotein synthesis (nuclear growth). In endo-

sperm and nucellar tissues, nuclear expansion occurs, but nuclear division fails to follow. Comparison of pH in these contrasting tissues is therefore of interest. Toward the end of the growing season, when pH determination by means of the range indicator method was attempted but while seed was still available, good stem material was already scarce. The pH of the nuclei of the cambial cells, $\pm$ 4.6, is markedly contrasted with that of the endosperm and nucellus, $\pm$ 6. These results are tentative only. Should further investigation confirm these divergent values, then pH may prove one significant factor in the fundamental processes of nuclear growth and chromosome division.

Preliminary tests with the oxidation-reduction indicator thionin (3) showed rapid penetration and at the time of entry differentiation of nucleoli, chromatin, and karyolymph. The first two stain clearly blue, as does lignin in a stem section; the latter light purple, as does cortical or pith parenchyma. This difference is later masked, and the nucleus as a whole appears purple. So far, however, it is the only indicator in the series used which distinguishes clearly between the component structures of a living nucleus.

## Summary

1. Development of the seed in *Echinocystis macrocarpa* follows the general outline given by KIRKWOOD for the Cucurbitaceae as a whole.

2. The cells and nuclei of endosperm and nucellus are visible to the naked eye in the full-grown but still unripened seed. The largest nuclei measure 150–200 $\mu$ in diameter. The nucleoli are extremely variable in number and outline.

3. The role of protein in maintaining the continuity of the chromatin strands is confirmed by qualitative experiments with the digestive enzyme trypsin. Disintegration of the nuclear reticulum by trypsin is inhibited by lanthanum chloride.

4. The reaction of Fehling's solution indicates the presence of reducing substances within the nucleus itself.

5. Actual increase in nuclear structural materials may be 3558-fold.

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## LITERATURE CITED

1. CASPERSSON, T., Über den chemischen Aufbau der Strukturen des Zellkernes. Skand. Arch. Physiol. 73. Suppl. 8. 1936.
2. CASPERSSON, T., and SCHULTZ, J., Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus. Proc. Nat. Acad. Sci. **26**:507–514. 1940.
3. CLARK, W. M., Studies on oxidation-reduction. Hygien. Lab. Bull. 151. 1928.
4. KIRKWOOD, J. E., Comparative embryology of the Cucurbitaceae. New York Bot. Gard. Bull. **3**:313–402. 1903–5.
5. KRATZER, J., Verwandschaftlichen Beziehungen der Cucurbitaceen auf Grund ihrer Samenentwicklung. Flora **109–110**:275–343. 1916–1918.
6. LEE, B. (edit: GATENBY), Microtomist's Vademecum. Philadelphia. 1928.
7. LLOYD, D. J., and SHORE, A., Chemistry of proteins. Philadelphia. 1938.
8. MAZIA, D., Enzyme studies on chromosomes. Cold Spring Harbor Symposia on Quantitative Biology **9**:40–46. 1941.
9. METZ, C. W., Structure of salivary gland chromosomes. Cold Spring Harbor Symposia on Quantitative Biology **9**:23–39. 1941.
10. PAINTER, T. S., On the synthesis of cleavage chromosomes. Proc. Nat. Acad. Sci. **26**:95–100. 1940.
11. PEARSALL, W. H., and PRIESTLEY, J. H., Meristematic tissue and protein isoelectric points. New Phytol. **22**:185–191. 1923.
12. SCOTT, FLORA M., Size of nuclei in the shoot of *Ricinus communis*. BOT. GAZ. **101**:625–636. 1940.
13. ———, Survey of anatomy, ergastic substances, and nuclear size in *Echinocystis macrocarpa* and *Cucurbita pepo*. BOT. GAZ. **104**:394–408. 1943.
14. SMALL, J., Hydrogen ion concentration in plant cells and tissues. Berlin. 1929.
15. TUNMANN, O., and ROSENTHALER, L., Pflanzenmikrochemie. Berlin. 1931.

# CYTOLOGICAL AND TAXONOMIC STUDIES IN THE GENUS BRODIAEA. II

MADELINE PALMER BURBANCK

## Introduction

In a previous paper (1), the chromosome numbers of twenty-four species and varieties of *Brodiaea* were reported, and taxonomic and cytological findings seemed to justify the separation of the plants into three genera—*Brodiaea*, *Dichelostemma*, and *Triteleia*. In some instances data on the plants were incomplete. Further observations have now been made, certain specific delimitations examined in more detail, and two additional species have been investigated and their chromosome numbers determined.

The bibliography on *Brodiaea* as given previously (1) has since been found to be incomplete. Following the classification of the monocotyledons as given by HUTCHINSON (6), FLORY and YARNELL (2) list chromosome numbers for *Brodiaea* and *Brevoortia* (*Dichelostemma*) under the Amaryllidales. The numbers given and the investigators cited are essentially the same as those already reviewed by the writer, with one addition. The diploid chromosome numbers of 12 for *Brodiaea uniflora* and 36 for *B. grandiflora* are listed, with YAMAMOTO given as the investigator. TISCHLER (7) also includes these species in his summary of *Brodiaea* chromosome numbers and gives the haploid numbers of 6 and 18, respectively, with YAMAMOTO as the authority for the counts. HOOVER (3) does not include *B. uniflora* and other South American "Brodiaeas" in his classification of North American Brodiaeas, and the species has not been investigated in connection with these studies. The number 36 for *B. grandiflora* does not agree with other reports on the species. Of the three species to which the name "*grandiflora*" has been applied, two have the diploid chromosome number of 32 and the third, 42 (1).

## Material and methods

The plants of *Brodiaea* reported upon earlier have been under observation for two additional years. In the fall of 1941 the majority of the corms were planted in the ground or in pots sunk in the ground in Moorestown, New Jersey, where they bloomed the following spring. Corms of *Dichelostemma volubile* and *D. ida-maia*, the hardiness of which was doubtful in this climate, and corms of *Triteleia laxa*, *T. laxa* Blue King, and *T. candida*, were taken to New York City and grown in the greenhouse on the top floor of Schermerhorn Extension, Columbia University. Also grown in the greenhouse were additional corms of *T. laxa*, *T. laxa* Blue King, and *D. ida-maia* purchased from Mr. CARL PURDY, Ukiah, California, and corms of *T. laxa* and *Bloomeria crocea* kindly sent by Dr. R. F. HOOVER of Yakima, Washington. Studies of the somatic and meiotic chromosomes of the greenhouse plants were made in the usual manner by root-tip sections and aceto-carmine smears of anthers. Root tips were fixed in either La Cour's 2 BE or Craf's solution. Slides made from the latter material tended to have clearer cytoplasm, but the actual outlines of the chromosomes were more definite in the material fixed in the 2 BE.[1]

[1] I wish to thank DR. S. F. TRELEASE and DR. M. M. RHOADES of the Botany Department of Columbia University for allowing me to use greenhouse space and laboratory facilities to carry on this investigation.

Late in the summer of 1942, when the corms were dormant, both the greenhouse plants and those grown outdoors in New Jersey were taken from the ground, sacked, and replanted in pots or in open ground outdoors in Springfield, Missouri. All species and varieties survived the winter of 1942–43, although some thrived better than others. No cytological studies were made of the plants while growing in Missouri, but records were kept of vegetative and flower characters.

Drawings were made at table level with the aid of a camera lucida. The magnification is approximately 1660. Drawings of mitotic chromosomes are from sectioned root-tip material and meiotic figures are from aceto-carmine smears of anthers.

## Further observations on Brodiaea Dichelostemma, and Triteleia

Supplementary data have been collected on the plant material analyzed in detail earlier (1). Within the genus *Brodiaea* in the limited sense, *B. purdyi* and *B. minor* continued to exhibit marked size differences when grown under natural conditions. The longer scapes and larger flowers of *B. purdyi* were very apparent. The color forms of *B. californica* were distinguishable in New Jersey, where the corms were kept separate. They were not kept separate in Missouri, and when in bloom, the flowers all appeared to be the same color.

The material received from PURDY under the names *Brodiaea capitata* and *B. capitata* var. *multiflora* was seen in bloom in 1942 and 1943. The violet flowers corresponded to the description of *Dichelostemma pulchellum* (the species determination given earlier based on synonomy), with one exception. Six anthers were not always present, the three smaller stamens opposite the outer segments being sometimes absent or only one or two present. There were no morphological differences between the flowers of the species and of the "variety," but the variety exhibited a slightly greater profuseness of bloom. Since the chromosome numbers are the same, the plants received as var. *multiflora* are probably merely a more vigorous form of *D. pulchellum*.

Previously it was noted (1) that the chromosomes of the species of *Dichelostemma* examined were characterized by smaller diameters than those of the *Triteleia* and *Brodiaea* chromosomes. Dr. WODEHOUSE examined the pollen of *Dichelostemma volubilis* and found the grains to be only about half the size of the pollen grains of *Triteleia* (*Brodiaea*) *laxa*, and with an entirely different pattern of the exine (private correspondence). This is further justification for division of the large group, *Brodiaea*, into separate units.

The corms of both *Brodiaea peduncularis* and *B. eastwoodii*, grouped together by HOOVER (5) into the one species, *Triteleia peduncularis*, bloomed in Missouri. Although only three flowering scapes were produced by *B. eastwoodii* and two by *B. peduncularis*, table 1 shows that *B. peduncularis* produced consistently longer pedicels, as maintained by PURDY. The very short pedicel, 2.1 cm., produced by one scape of *B. peduncularis* was an exception, as can be seen by the averages. The flowers of the two plants were slightly different, although both fitted the description of *T. peduncularis* as given by HOOVER (5). The scapes of *B. peduncularis* were more slender than those of *B. eastwoodii*. The perianths of *B. peduncularis* were white, each segment with a bluish gray-green line down the

back which gave a bluish tinge to the inner surface; the anthers were white or sometimes bluish and somewhat reduced in size. The perianths of *B. eastwoodii* were creamy white on the inner surface, with a green line down the back of each segment; the anthers were white. On the basis of these observations, the chromosome numbers of 2n = 14 for *B. eastwoodii* and 2n = 28 for *B. peduncularis*, and the discussion presented earlier (1),

TABLE 1

COMPARISON OF B. EASTWOODII AND B. PEDUNCULARIS

| Species | No. of flowers | Length of scape (cm.) | Pedicel length (cm.) | |
|---|---|---|---|---|
| | | | Average | Range |
| | | Scape I | | |
| B. eastwoodii | 17 | 39.0 | 3.62* | 2.2–4.4 |
| B. peduncularis | 5 | 19.5 | 4.88† | 2.1–6.2 |
| | | Scape II | | |
| B. eastwoodii | 11 | 31.5 | 3.06* | 1.5–4.7 |
| B. peduncularis | 7 | 20.0 | 5.97† | 3.2–7.8 |

* Average 3.34. † Average 5.43.

it seems possible that there is a form of *T. peduncularis* sufficiently distinct as regards morphological characters and chromosome number as to be considered a named variety of the species.

Consideration of further findings in the *Triteleia laxa* complex will be taken up in detail later. Figure 1 shows the 42 meiotic chromosomes of *B. laxa* Blue King which were not clearly distinguished during earlier study. *Triteleia crocea* bloomed in Missouri, and the species determination formerly based on synonomy only was found to be correct. The material labeled *T. hendersoni* bloomed in New Jersey but produced purple flowers resembling those of *B. purdyi*. It is possible that corms of *T. hendersoni* have not been used in these studies and that the data reported for *T. hendersoni* properly belong to some other species.

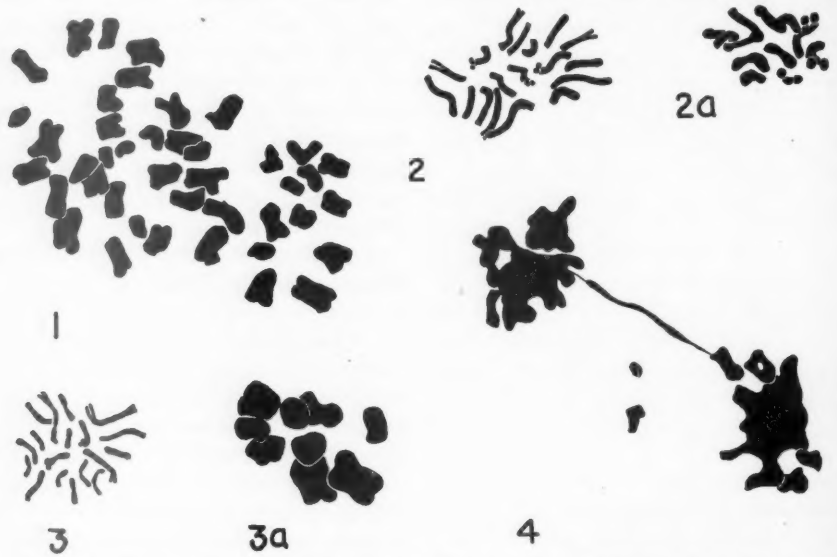
### Triteleia laxa complex

Earlier findings (1) showed that plants called by Purdy *Brodiaea laxa*, *B. laxa* Blue King, and *B. candida* could be separated from one another on the basis of chromosome numbers. Certain morphological differences were noted, but in his discussion of *Triteleia laxa*, Hoover (5) maintains that there is such great variation of some characters among the forms of *T. laxa* and such constancy in essential characters that it should be regarded as a single taxonomic unit. Thus *B. laxa*, *B. laxa* Blue King, *B. candida*, and other similar forms should all be called *T. laxa*. By keeping individual records of a greater number of plants comprising this "*Triteleia laxa* complex," it was hoped that further conclusions could be reached. Hoover kindly sent corms of a form of *T. laxa* found in the San Joaquin Valley, California, and additional material of *B. laxa* and *B. laxa* Blue King was purchased from Purdy. Table 2 gives a summary of some of the records kept on these plants grown in the greenhouse in New York City in 1941–42.

The material received from Hoover as *Triteleia laxa* bloomed sparingly but fitted his description and resembled the plants of *B. candida*, *B. laxa*, and *B. laxa* Blue King with minor exceptions as noted later. Studies of the mitotic and meiotic chromosomes, however, revealed that n = 9 and 2n = 18 (fig. 2, *2a*), numbers entirely different from those found for other members of this complex.

The differences in vegetative charac-

ters and flower morphology noted earlier within the *T. laxa* complex continued to exist under more careful analysis, but certain characters were common to all the forms. The leaves of *B. candida* were yellow-green rather than the darker green of the other plants. The leaves of *B. laxa* Blue King were wider than those of *B. laxa* on the average, but this did not hold true in every case. There were only slight differences in size and shape of the corollas of the four groups of plants. The color of the corolla varied from lavender to deep purple, but no one form had a distinct and characteristic color. Some pedicels formed an angle with the corolla tube, some did not. One flowering scape of *B. candida* produced flowers which faced all in one direction and were approximately horizontal. This may be the condition upon which PURDY (private communication) bases the separation of *B. candida* from *B. laxa*. The position of the pistil, whether central or along the lower side of the flower, was variable in all four groups. The differences in size and shape of the anthers appears significant. *B. candida* and *T. laxa* had anthers 2.5–4.0 mm. in length which were blunt and rounded at the apex. *B. laxa* and *B. laxa* Blue King had anthers 4.0–7.0 mm. in length, and they tapered upward to a point. Thus in size and shape there is a consistent difference in the morphology of the anthers. In the species description of *T. laxa* (5), HOOVER states that the anthers are 2–5 mm. long. The time of flowering was earlier for *B. candida* and *T. laxa* than for *B. laxa* and Blue King (table 2). Only two corms of Blue King bloomed in February and one in March, the height of bloom being in April. *T. laxa* bloomed late in February, *B. candida* early in March, and *B. laxa* in March and April. The characters common to all forms of




FIGS. 1–4.—Fig. 1, *B. laxa* Blue King, anaphase I. Fig. 2, *T. laxa*, root-tip metaphase. Fig. 2*a*, same, metaphase II. Fig. 3, *Bl. crocea*, root-tip metaphase. Fig. 3*a*, same, metaphase I. Fig. 4, *B. laxa* Blue King, telophase I.

*T. laxa*, and which determine the species, include the attachment of stamens at two different levels with filaments of about equal length, and perianth blue with the tube attenuate at base and ranging in length from 22 to 38 mm.

Since a variation of the chromosome number for *B. laxa* was noted earlier, an attempt was made to study both somatic and meiotic chromosomes of each plant under observation and note any correlation between chromosome morphology and vegetative and flower characters. of telophase II. Bridging and lagging at both telophase I and II were seen occasionally in the anther smear material of two of the three plants and in two additional plants for which the meiotic count was not determined.

Similar meiotic irregularities were noted in the Blue King material. Bridging as shown in figure 4 was especially common in the plant from which the pictured material was taken. A temporary slide made from an anther of the same plant (fig. 4) contained 31 normal telo-

TABLE 2

DATA ON TRITELEIA LAXA COMPLEX

| Species | No. of corms | No. of plants with flowers | Appearance of first flower (1943) | Average | | Range | | Chromosome number (2n) | No. of plants with meiotic irregularities |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Leaf width (mm.) | Anther length (mm.) | Anther length (mm.) | Corolla length (cm.) | | |
| B. laxa | 31 | 15 | 3/11–4/25 | 7.9 | 5.3 | 4.0–6.5 | 2.5–3.8 | 28–30 | 5 |
| B. laxa Blue King | 33 | 19 | 2/9 –5/3 | 11.5 | 5.9 | 4.5–7.0 | 2.6–3.8 | 42 | 6 |
| T. laxa | 21 | 2 | 2/18–2/28 | ..... | 3.0 | 3.0 | 2.4–3.2 | 18 | 0 |
| B. candida | 13 | 4 | 3/1 –3/11 | ..... | 3.5 | 2.5–4.0 | 2.2–3.7 | 48 | 0 |

The data on this are not complete, but no striking correlations were noted. Of six plants of *B. laxa* for which the somatic chromosome number was determined, three had 30 chromosomes, two had 28, and one appeared to have a variable number, always slightly above 30. Meiotic chromosome counts of three other plants of this form revealed 14 chromosomes in two cases and 14 chromosomes plus extra fragments in the third. The extra fragments, usually but not always visible and one, two, or three in number, were especially apparent at metaphase I, when they lay in the cytoplasm at some distance from the metaphase plate. Their ultimate fate could not be determined. In only one instance was a bit of chromatin seen isolated from the four chromatin masses characteristic phase stages and 44 telophase stages in which there were fragments or bridging or both. No irregularities were observed in the material of *T. laxa* and *B. candida* examined.

In the Chicago greenhouses, neither *B. laxa* nor *B. laxa* Blue King set seed. In New York, seven selfings of *B. laxa* resulted in capsules of seed. (Under the term "selfing" is included both the use of pollen from the same flower and of pollen from another flower produced on the same scape.) One selfing and three attempts at crossing two different plants of *B. laxa* yielded no seed. Three selfings of Blue King, two of the same plant, produced seeds, but eight selfings yielded no seed. Six attempts at crossing two different plants of Blue King yielded no seed. Using both *B. laxa* and Blue King as

pollen parents, thirteen crossings were attempted between the plants with negative results. Three attempts to cross *B. candida* and *B. laxa* produced no seed. The cytological data on these plants are not complete enough to determine whether the varying chromosome number of *B. laxa* and the meiotic irregularities of both *B. laxa* and Blue King influence the ability to produce seed. Apparently both forms can reproduce by means of seeds, and crossing between the two is rare or impossible.

Dr. WODEHOUSE kindly examined some of the pollen of the *Brodiaea* plants grown in New York City. He found that the pollen grains of *B. laxa* and *B. laxa* Blue King were almost alike. They differed only in the slightly larger size of the Blue King grains, 70.7 $\mu$ as compared with 51.6 $\mu$ for *B. laxa*, and the pattern of the exine, which was finely reticulate in the former and merely pitted in the latter.

The findings outlined in the foregoing paragraphs confirm HOOVER's statement that *T. laxa* is a variable species. Whether it is a single species, or whether it contains forms that should be given varietal or species rank, cannot be determined without further field work and cytological analysis of material collected. Such a question cannot be settled by laboratory and greenhouse work and relatively small numbers of plants. *B. laxa* Blue King appears to be a slightly more vigorous form of *B. laxa*. The only "good" characters found in this study upon which separation could be based are the size and shape of anthers, the time of flowering, and the chromosome number. It is unusual to have such 2n chromosome numbers as 18, 28-30, 42, and 48 occur within a single species. Six would seem to be the basic number of such a series. These numbers and the meiotic irregularities observed suggest that the "species" *T. laxa* is a complex still in the evolutionary process of becoming a species.

### DICHELOSTEMMA VOLUBILE × D. IDA-MAIA

A capsule of seed was reported (1) following the pollination of a flower of *Dichelostemma volubile* with pollen from *D. ida-maia*. Planted outdoors the following fall, these seeds did not germinate. Forty-one further attempts at cross-pollination between these two species failed to yield a single seed. It is concluded that a cross is not possible and that the earlier seeds were not actually the product of such a cross or were not viable. A misstatement concerning *D. venustum* in this connection was made earlier. *D. venustum* is not a hybrid between *D. volubile* and *D. ida-maia* but has been claimed to be a hybrid between *D. congestum* and *D. ida-maia* (4).

### BLOOMERIA CROCEA

HOOVER (5) has stressed the similarities between *Triteleia* and *Bloomeria*. A study of the chromosomes of *B. crocea* does not bear out this close association. The mitotic chromosome number was 18 and the meiotic number 9 (fig. 3, 3*a*). Individual chromosome morphology was not distinct enough to be significant, but the small diameters of the mitotic chromosomes and the number 18 suggest a closer relationship with *Dichelostemma* than with either *Triteleia* or *Brodiaea*. WODEHOUSE found the pollen grains of *Bloomeria crocea* to be quite different from those of *Triteleia*, *Brodiaea laxa*, and *Dichelostemma volubilis*, both in shape and in the texture of the exine.

## Summary

1. The species determinations of *Dichelostemma pulchellum* and *Triteleia*

*crocea* were confirmed by examination of fresh flowers. The identity of the material considered as *Triteleia hendersoni* is doubtful.

2. The existence of a variety of *Triteleia peduncularis*, based on pedicel length, flower color, chromosome number, and perhaps distribution, is suggested.

3. *Triteleia laxa* is considered a species complex made up of many forms. Somatic chromosome numbers include 18, 28, 30, 42, and 48. Reconsideration of the species delimitation seems indicated.

4. *Dichelostemma volubile* and *D. idamaia* are not readily crossed.

5. *Bloomeria crocea* has 18 mitotic and 9 meiotic chromosomes.

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LITERATURE CITED

1. BURBANCK, MADELINE P., Cytological and taxonomic studies in the genus *Brodiaea*. BOT. GAZ. 103: 247–265. 1941.
2. FLORY, W. S., and YARNELL, S. H., A review of chromosome numbers in the Hemerocallideae, Alstroemeriales, and Amaryllidales. Herbertia 4: 163–181. 1937.
3. HOOVER, R. F., A definition of the genus *Brodiaea*. Bull. Torr. Bot. Club 66: 161–166. 1939.
4. ———, The genus *Dichelostemma*. Amer. Mid. Nat. 24: 463–476. 1940.
5. ———, A systematic study of *Triteleia*. Amer. Mid. Nat. 25: 73–100. 1941.
6. HUTCHINSON, J., The families of flower plants. II. Monocotyledons. London. 1934.
7. TISCHLER, G., Pflanzliche Chromosomen-Zahlen. Tab. Period. 12: 57–115. 1937.

# NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF CENTAUREA PICRIS[1]

JOHN C. FRAZIER

## Introduction

This is the third in a series of reports on the growth habits of noxious perennial weeds of central United States, the work being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress. The present study has to do with Russian knapweed, *Centaurea picris* Pall. (*C. repens* L.), a noxious weed of potential significance rivaling that of field bindweed and hoary cress in this area.

## Environmental conditions and methods

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the profile of which was described by FRAZIER (3). No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1942 compared with the long-time average of these factors are given in table 1. The summer of 1942 had temperatures of 100° F. or higher for 5 days, as compared with 38 such days in 1937—the year the comparable study was made on field bindweed, and 15 such days in 1941—the year hoary cress was investigated. The average annual number of days having

[1] Contribution no. 454, Department of Botany, Kansas Agricultural Experiment Station.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

this temperature during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1942 was 3.81 inches, as compared with the monthly average of 2.86 for these months during the 83-year period, 1858–1940. Much of the precipitation for the first 10 months of 1942 fell as torrential rains. There was a 24-hour period in May

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1942

| MONTH | TEMPERATURE | | PRECIPITATION | |
|---|---|---|---|---|
| | Mean* | 85-year average (1856–1940) † | Total* | 83-year average (1858–1940) † |
| Jan. | 30.2 | 27.0 | 0.64 | 0.75 |
| Feb. | 32.7 | 31.5 | 0.93 | 1.14 |
| Mar. | 44.9 | 42.5 | 1.61 | 1.48 |
| Apr. | 60.4 | 54.5 | 1.77 | 2.67 |
| May | 63.4 | 64.6 | 6.00 | 4.31 |
| June | 73.2 | 74.5 | 11.48 | 4.50 |
| July | 80.8 | 79.5 | 2.19 | 4.25 |
| Aug. | 77.7 | 77.5 | 3.85 | 3.89 |
| Sept. | 67.1 | 69.3 | 7.45 | 3.44 |
| Oct. | 59.0 | 56.5 | 2.20 | 2.16 |
| Annual mean or total | 55.6 | 54.2 | 41.14 | 30.97 |

* Meteorological data from U.S. Weather Bureau, Kansas section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

with 1.75 inches; two in June with 1.77 and 2.85 inches, respectively; and one in September with 3.25 inches.

METHODS.—The plants were grown on a small plot of land free of both noxious weeds and sodium chlorate. The soil is a fairly typical Geary silt loam, representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

The first planting was made April 11, 1942. Several seedlings emerged on April 22. A plant removed April 29 was designated no. 1. It illustrates a seedling 1 week after emergence. A second planting was made April 18. In each planting, twenty seeds were placed $\frac{3}{8}$ inch below the surface at each of twenty-one points so spaced that seven of the plantings were 13 feet apart and the remainder were separated by 5–8 feet. Emergence of the second planting began April 27. On April 30, seedlings emerged at sixteen of the twenty-one points. These sixteen seedlings were retained but all others were removed, including those emerging after that date. All plants used except no. 1 emerged on April 30. The area was kept free of all other vegetation, so that the only competition for water and nutrients, if any existed, was among the knapweed plants.

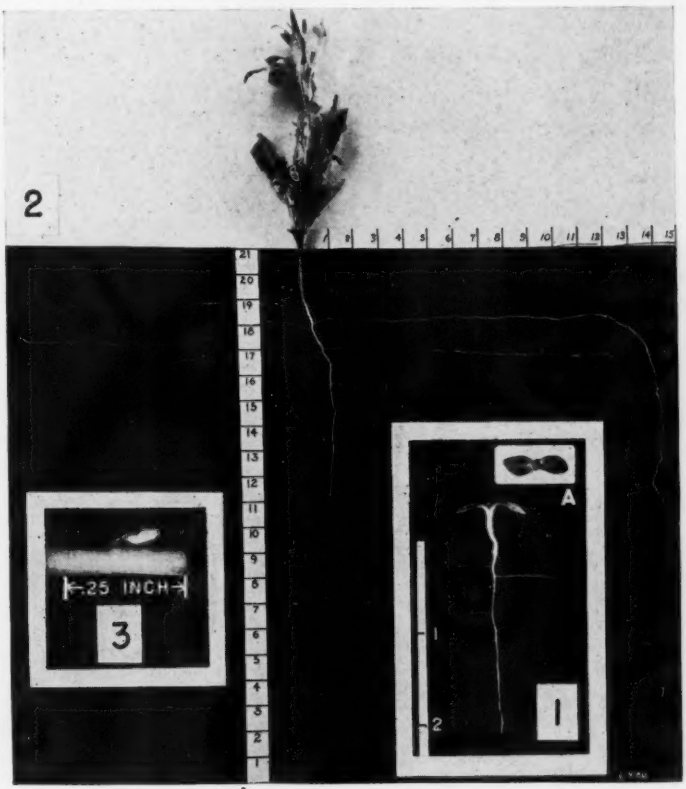
The root systems were excavated by a modification of the trenching method developed by WEAVER (7) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupied the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 4, and 5 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a great portion of them was secured.

## Observations

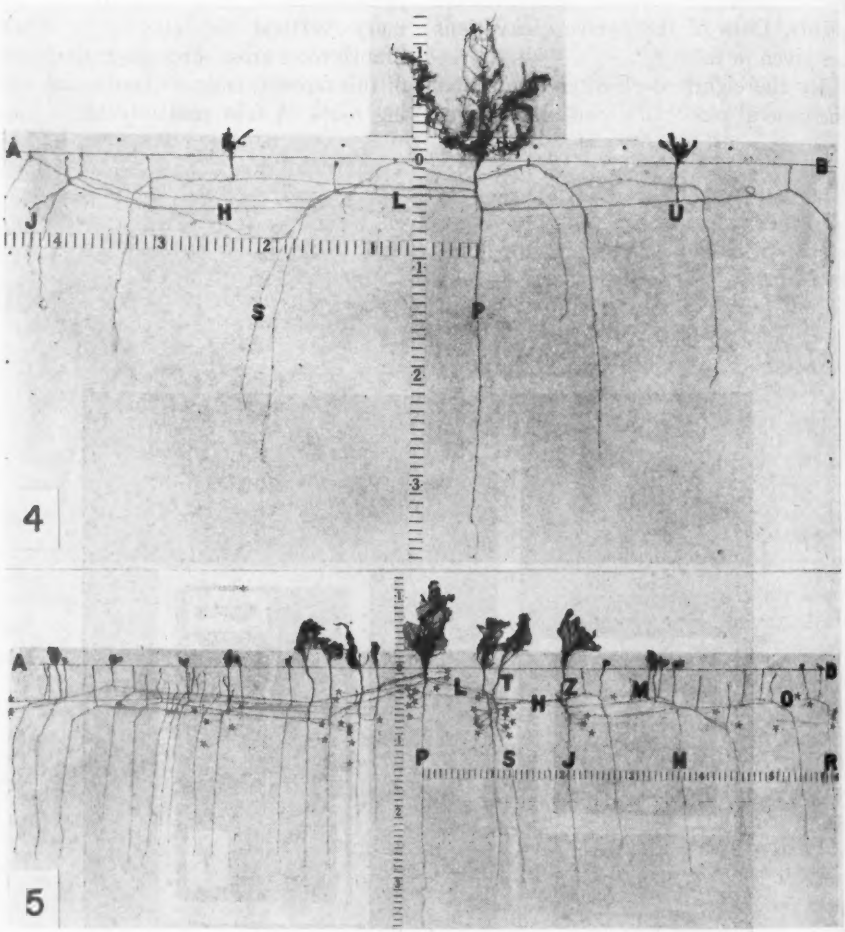
Eight plants were excavated weekly between April 29 and July 16, except for the second, fifth, and tenth weeks after emergence, and plants 9, 10, and 11 excavated August 6, September 17, and in early November, 1942, respectively. A twelfth plant was excavated in mid-September, 1943. Figures 1, 2, 4, and 5 show the vertical penetration and the

radial spread of roots of representative plants. Data of the twelve excavations are given in table 2.

By the eighth week after emergence, the general plan of the root system was beginning to be established (fig. 2). Plant no. 10, taken the twentieth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and nutrients, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots of the first order (fig. 4*L*). These grew horizontally 12–36 inches and then turned down and were from that point vertical taproots, desig-




FIGS. 1–3.—Stages in development of plants of Russian knapweed. Fig. 1, seedling 1 week after emergence; *A*, view of cotyledons from above. Fig. 2, young plant 8 weeks after emergence showing permanent lateral turning down to become secondary vertical root. Fig. 3, root-borne stem bud, ×3, on primary permanent root of plant 11 weeks after emergence. Scales in inches.




FIGS. 4, 5.—Fig. 4, complete plant 20 weeks after emergence (*A*–*B* is ground line). *P*, primary vertical; *L*, permanent lateral of first order; *S*, secondary vertical of first order; *H*, permanent lateral of second order; *J*, secondary vertical of second order; *U*, rhizome arising from lateral root. Fig. 5, approximately 60% of growth attained by one plant 7 months after emergence, arranged to show place relationship of parts essentially as in the soil. Stars indicate points from which laterals have been removed for clarity. *M*, permanent lateral of third order; *N*, secondary vertical of third order; *O*, permanent lateral of fourth order; *R*, secondary vertical of fourth order; *T*, *Z*, heavy rhizome and shoot development on or near juncture of permanent lateral and secondary vertical roots. Scales in feet and inches.

nated secondary vertical roots[3] (fig. 4*S*). The permanent lateral roots of any order, which are usually smallest in diameter at their origin, were always in the surface foot, more commonly in the upper 7 inches of the soil. The lateral-vertical roots of the first order gave rise to branch roots from any part of the horizontal or being away from the primary vertical, and turned down to form secondary verticals of the second order (fig. 4*J*). This development was repeated many times during the growing season to give rise to lateral-vertical roots of the third, fourth, and even higher orders (fig. 5). It is by such series of permanent laterals

TABLE 2

RATE AND NATURE OF DEVELOPMENT* AFTER SEEDLING EMERGENCE OF ROOTS OF RUSSIAN KNAPWEED. MANHATTAN, KANSAS, 1942

| Plant no. | Weeks after emergence | Maximum vertical penetration (inches) | Maximum radial spread (inches) | Plant condition |
|---|---|---|---|---|
| 1 | 1 | 2¼ | ⅝ | Typically shaped cotyledons (fig. 1, 1A) |
| 2 | 3 | 9½ | 3¼ | First true leaves |
| 3 | 4 | 14 | 5¼ | Additional true leaves beginning to form rosette; cotyledons lost |
| 4 | 6 | 17⅛ | 7½ | Additional true leaves |
| 5 | 7 | 19⅝ | 9 | Rosette of leaves well developed |
| 6 | 8 | 21 | 14½ | Lateral roots beginning to grow downward at tip to become secondary verticals (fig. 2) |
| 7 | 9 | 17½ | 12 | Several secondary vertical roots grown downward approximately 12 inches |
| 8 | 11 | 31½ | 22 | First root-borne stem buds on lateral roots 16–17 inches from primary vertical root (if underground, these develop into rhizomes) |
| 9 | 14 | 31¼ | 22 | Rhizomes on lateral roots giving rise to emerging leafy shoots |
| 10 | 20 | 44 | 59½ | Many rhizomes reaching soil surface and giving rise to well-developed shoots (fig. 4) |
| 11 | 28 | 45½ | 78 | Root development including permanent lateral and secondary verticals through fifth order (fig. 5) |
| 12 | 72 (1943) | 10½ ft. | 11–12 ft. (at least) | Secondary vertical roots apparently penetrating deeper than primary verticals and larger in diameter, particularly in first 12–24 inches below bend where lateral root becomes secondary vertical |

* First flowering occurred July 7, 1943.

vertical growth. Most of these are small feeding roots. However, at or just below the bend where the permanent lateral of the first order becomes a secondary vertical root, permanent laterals of the second order developed (fig. 4*H*). These extended horizontally 6–22 inches from the point of origin, the general direction that horizontal spreading of the plant is accomplished. If more than one permanent lateral arose from the place of origin of such roots of the second or higher orders, they radiated from the point of origin; but, in general, all maintained growth away from the primary vertical. The result was that the plant occupied a much higher proportion of the ever-increasing area, somewhat circular in shape, than it would have otherwise. Concurrent studies indicated that plants

[3] The horizontal and vertical phases of these roots are included in the term lateral-vertical. The vertical phase is considered to begin where the slender lateral enlarges markedly.

growing under conditions of severe competition made less lateral development in unit time.

All shoot development, except that from the plumule, was derived from root-borne stem buds. These developed on any part of the permanent root system (that is, the permanent laterals and the two types of vertical roots). Eleven weeks after first seedling emergence, a number of these buds had formed on or near the bend between the permanent lateral and the secondary vertical roots. A few also developed on the permanent laterals. One of the latter is shown in figure 3. By 14 weeks after seedling emergence such buds had produced rhizomes, some of which had grown to a length of 2 inches. Two of them initiated shoots upon reaching the surface. By 20 weeks after seedling emergence, shoots from rhizomes were well developed (fig. 4). The location of the most extensive shoot development from root-borne stem buds was at or near the bend between the lateral root of any order and its vertical phase (fig. 5*T*, *Z*). Infrequently, shoots not subtended by a vertical root were found along the permanent laterals (fig. 4*U*). Regardless of where these buds formed, they gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

Twenty-eight weeks after it had emerged as a seedling, a plant (fig. 5) had sent its primary vertical root to a depth of $45\frac{1}{2}$ inches and had produced sixteen permanent lateral roots of the first order. The latter were arranged radially around the primary vertical. Fourteen of these laterals had developed secondary vertical roots. Eleven of the fourteen had given rise to lateral-secondary verticals of the second and higher orders. Seven of the eleven are shown in figure 5. There were twenty-eight regions of shoot development on the fourteen lateral roots which had developed secondary verticals. The shoot development of these twenty-eight regions came from seventy-one rhizomes, each of which had developed from a root-borne stem bud. There were no rhizomes or root-borne buds on the two laterals which had not formed secondary vertical roots. The growth attained 28 weeks after emergence was so carefully removed that practically all the plant was recovered. Approximately 60% of the growth constituting the plant was arranged to show the place relationship of these parts essentially as they were in the soil (fig. 5).

## Discussion

The rate of growth of plants of Russian knapweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable non-competitive situation.

The observations of BALL and ROBBINS (1) on the general nature of the root system of this plant agree in general with the present findings. However, BALL and ROBBINS consider the horizontal axes of the root system as "creeping, perennial rootstocks," on which "close observation . . . . shows small, narrow, appressed scale leaves, arranged alternately at regular intervals." In the study here reported, numerous observations, both macroscopic and microscopic, show the horizontal axes to be roots and not underground stems, as the term rootstock used by BALL and ROBBINS implies. ROGERS (5) considered the horizontal axes to be roots. Buds (fig. 3) do arise adventitiously at irregular intervals on the horizontal roots, and less commonly on the upper portions of their vertical extensions (secondary vertical roots), but they are not subtended by scale

leaves. The horizontal axes are roots, not rootstocks (underground stems), in field bindweed and hoary cress—weeds with a root system identical in general form with that of Russian knapweed. KENNEDY and CRAFTS (4) reported this fact for field bindweed, SIMONDS (6) for hoary cress, and FRAZIER (2, 3) confirmed the finding.

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (4) for field bindweed and by FRAZIER (3) for hoary cress that these variations result from differences in the supplies of soil moisture and plant nutrients.

## Summary

1. Plants of Russian knapweed, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied from the seedling stage through 72 weeks of growth.

2. The root system of well-established plants consisted of the original root (primary vertical), one to many permanent lateral roots, and their vertical extensions (secondary vertical roots).

3. The plants spread horizontally by series of permanent lateral roots. The permanent laterals of the first order arise on the primary vertical root. Unless injury or too severe competition prevents, successive orders of permanent laterals arise at or near the bend where a permanent lateral of the preceding order turns down to become a vertical (secondary vertical root).

4. The plants had spread radially $6\frac{1}{2}$ feet in one growing season, and 11–12 feet by the end of the second season, at which time several vertical roots had reached a depth of $10\frac{1}{2}$ feet.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which produce either leafy shoots directly (if at the ground line), or rhizomes (if below ground) which in turn give rise to leafy shoots. These buds arose in greatest abundance at or near the bend separating the permanent lateral of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

6. The general type of development is the same as that of field bindweed and hoary cress. The rate of development of knapweed appeared to be about the same as that of hoary cress for the first 72–75 weeks. After the first 8–10 weeks following emergence, both plants developed at a somewhat slower rate than bindweed. Bindweed flowered during the first season of growth, while hoary cress and knapweed did not flower until the second season.

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## LITERATURE CITED

1. BALL, W. S., and ROBBINS, W. W., Russian knapweed. California Dept. Agr. Monthly Bull. 20:666–668. 1931.
2. FRAZIER, J. C., Nature and rate of development of root system of *Convolvulus arvensis*. BOT. GAZ. 104:417–425. 1943.
3. ———, Nature and rate of development of root system of *Lepidium draba*. BOT. GAZ. 105:244–250. 1943.
4. KENNEDY, P. B., and CRAFTS, A. S., The anatomy of *Convolvulus arvensis*, wild morning-glory or field bindweed. Hilgardia 5:591–622. 1931.
5. ROGERS, C. F., Canada thistle and Russian knapweed and their control. Colorado Agr. Exp. Sta. Bull. 348. 1928.
6. SIMONDS, A. O., The anatomical development of *Lepidium draba*. Jour. Agr. Res. 57:917–928. 1938.
7. WEAVER, J. E., Root development of field crops. McGraw-Hill, New York. 1926.

# EFFECT OF VARYING NUTRITIONAL TREATMENTS ON GROWTH AND RUBBER ACCUMULATION IN GUAYULE

JAMES BONNER

## Introduction

Preliminary experiments conducted in 1941 suggested that rubber accumulation in guayule (*Parthenium argentatum* Gray) plants might be influenced significantly by the nature and amount of available nutrients. Two experiments designed to explore this possibility were conducted during 1942–43. In the first, plants were supplied with thirty-eight different nutrient solutions of varying anion or cation composition planned according to a triangular system (2). In the second experiment, young plants were supplied with nitrogen, either as nitrate, as ammonium, or as mixtures of the two forms. Each experiment lasted through an entire growing season of 8.5 months, and since they were conducted outdoors, the plants were exposed to the changing environments of summer, fall, and winter. In each case two harvests were made, one at the end of the summer, when rubber content of the plants was low, and one the following spring, when rubber content had reached its maximum for the season.

## Material and methods

The planting stock consisted of nursery seedlings[1] grown by the American Rubber Producers, Inc., and supplied by the U.S. Forest Service. The plants had the tops removed close to the crown and were without leaves. In addition, they were small and weighed an average (dry) of 60 mg. per plant. During the first week in June this material was planted in 4-mesh gravel contained in 1.5-gallon cans provided with drainage and which had been previously painted with a nontoxic asphaltum paint. Three plants were placed in each of approximately 1000 cans. After 7–10 days, when new shoot growth had begun to appear, one plant was removed from each can, leaving the two most uniform. On June 15, 1942, 604 containers of plants selected for uniformity were chosen for initiation of the first experiment.

[1] Owing to critical shortage of guayule plants in the spring of 1942, the U.S. Forest Service was able to supply only cull nursery stock.

## Experiment I

NUTRIENT SOLUTIONS.—This experiment was divided into two parts, one in which nutrient solutions of varying anion composition were applied and a second in which solutions of varying cation composition were applied. Six stock solutions, whose compositions are given in table 1, were prepared (2). Three of these stock solutions each contained one major anion but had similar composition as to cations. The other three each contained one major cation but had similar composition as to anions. The three anion solutions were combined by ninths to give nineteen of the fifty-five possible combinations, while the three cation solutions were similarly combined to give nineteen further combinations. Figure 1*A* shows the positions in the triangles occupied by the thirty-eight solutions. Table 2 shows the proportions of the six stock solutions used in making up the thirty-eight nutrient solutions. The anion solutions maintain constant ratios between cations (table 1).

The stock solutions were made with chemicals of technical or U.S.P. grade.

All solutions were made with tap water, and the plants were watered with tap water. Nutrient was applied at the rate of 500 cc. per culture three times per week, and the plants were further watered as required.

EXPERIMENTAL DESIGN.—The anion and cation treatments, each including nineteen nutrients, were tested in each of four plots. In each plot the nineteen treatments were randomized, and each treatment was tested with four replicate cultures. Thus, any one treatment was tested in four plots, each containing four cultures (each of two plants), or a total of thirty-two plants per treatment. The data were treated throughout by the analysis of variance, and summaries of the treatments appear in the tables. The cation and anion portions of the experiment were set up in separate but adjoining locations and are not strictly comparable, as is evident from the data.

HARVEST OF SEPTEMBER 15.—On September 15, after 3 months of treatment, half the cultures were harvested. Each treatment was represented in this harvest by sixteen plants; that is, by two plants from each of two cultures from each of four plots. The plants were washed free of gravel, dried rapidly at 65° C., and weighed (the leaves separately from the woody portion of stems and roots). This woody portion was then ground in a Wiley mill and analyzed for rubber and resin by the solvent-extraction method.

Figure 2*A* gives data on the dry weight of stems and roots for the nineteen anion treatments, while figure 2*B* gives data concerning the leaf dry weights for the same treatments. Results of analysis-of-variance treatments for the two sets of data are given in table 3. Significant differences in dry weights of both the woody portion and

TABLE 1

CONCENTRATIONS OF STOCK SOLUTIONS I–VI IN MILLIEQUIVALENTS OF INDIVIDUAL IONS PER LITER*

| Salt | I $NO_3$ | II $SO_4$ | III $H_2PO_4$ | IV Mg | V K | VI Ca |
|---|---|---|---|---|---|---|
| $KNO_3$ | 4.5 | | | | 4.5 | |
| $Ca(NO_3)_2$ | 12.0 | | | | | 12.0 |
| $Mg(NO_3)_2$ | 9.0 | | | 9.0 | | |
| $K_2SO_4$ | | 4.5 | | | 4.5 | |
| $CaSO_4$ | | 12.0 | | | | 12.0 |
| $MgSO_4$ | | 9.0 | | 9.0 | | |
| $KH_2PO_4$ | | | 4.5 | | 4.5 | |
| $Ca(H_2PO_4)_2$ | | | 12.0 | | | 12.0 |
| $MgHPO_4$ | | | 9.0 | 9.0 | | |

* Each stock solution contained in addition: 0.5 p.p.m. B as $H_3BO_4$, 0.5 p.p.m. Mn as $MnSO_4$, 0.1 p.p.m. Zn as $ZnSO_4$, 0.02 p.p.m. Cu as $CuSO_4$, 0.01 p.p.m. Mo as $H_2MoO_4$, and 1.0 p.p.m. Fe as ferrous tartrate.

TABLE 2

RATIOS OF STOCK SOLUTIONS I–VI USED IN MAKING UP NUTRIENT SOLUTIONS FOR EXPERIMENT I. FIGURES ARE PARTS OF INDIVIDUAL STOCK SOLUTIONS PER NINE PARTS OF NUTRIENT

| Nutrient solution | I $NO_3$ | II $SO_4$ | III $H_2PO_4$ | Nutrient solution | IV Mg | V K | VI Ca |
|---|---|---|---|---|---|---|---|
| 1 | 9 | 0 | 0 | 21 | 9 | 0 | 0 |
| 2 | 7 | 1 | 1 | 22 | 7 | 1 | 1 |
| 3 | 6 | 3 | 0 | 23 | 6 | 3 | 0 |
| 4 | 6 | 0 | 3 | 24 | 6 | 0 | 3 |
| 5 | 5 | 2 | 2 | 25 | 5 | 2 | 2 |
| 6 | 4 | 4 | 1 | 26 | 4 | 4 | 1 |
| 7 | 4 | 1 | 4 | 27 | 4 | 1 | 4 |
| 8 | 3 | 6 | 0 | 28 | 3 | 6 | 0 |
| 9 | 3 | 3 | 3 | 29 | 3 | 3 | 3 |
| 10 | 3 | 0 | 6 | 30 | 3 | 0 | 6 |
| 11 | 2 | 5 | 2 | 31 | 2 | 5 | 2 |
| 12 | 2 | 2 | 5 | 32 | 2 | 2 | 5 |
| 13 | 1 | 7 | 1 | 33 | 1 | 7 | 1 |
| 14 | 1 | 4 | 4 | 34 | 1 | 4 | 4 |
| 15 | 1 | 1 | 7 | 35 | 1 | 1 | 7 |
| 16 | 0 | 9 | 0 | 36 | 0 | 9 | 0 |
| 17 | 0 | 6 | 3 | 37 | 0 | 6 | 3 |
| 18 | 0 | 3 | 6 | 38 | 0 | 3 | 6 |
| 19 | 0 | 0 | 9 | 39 | 0 | 0 | 9 |

the leaves produced in the nutrient treatments are apparent. Of the deficient plants, those which received low nitrate were the smallest. These were stunted, possessed few and yellow leaves, and flowered meagerly. A less well-marked response to phosphate deficiency was evident. Phosphate-deficient plants showed a slight tendency to anthocyanin formation. No marked response to sulphate deficiency took place under the conditions the same conditions showed a rapid increase in rubber concentration during the first 2 weeks of September. Rubber concentration, though low, was significantly affected by anion nutrition, in particular by the nitrate level. Plants which received either much or very little nitrate contained significantly lower rubber concentrations than plants supplied with nitrate at an intermediate level. No specific influence of phosphate

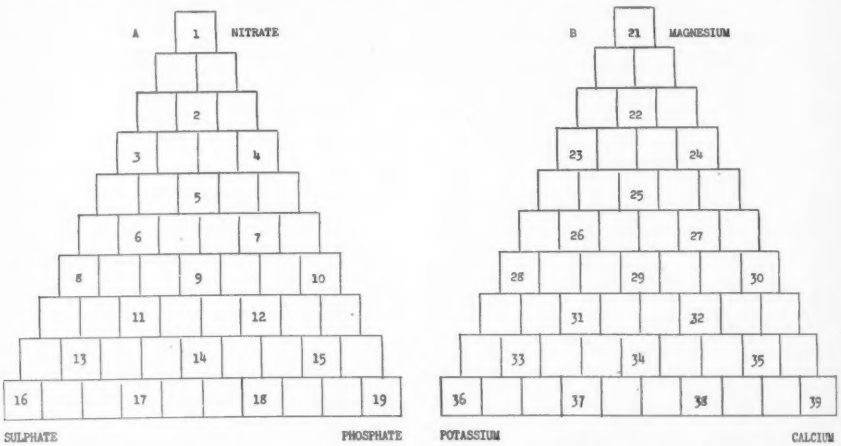


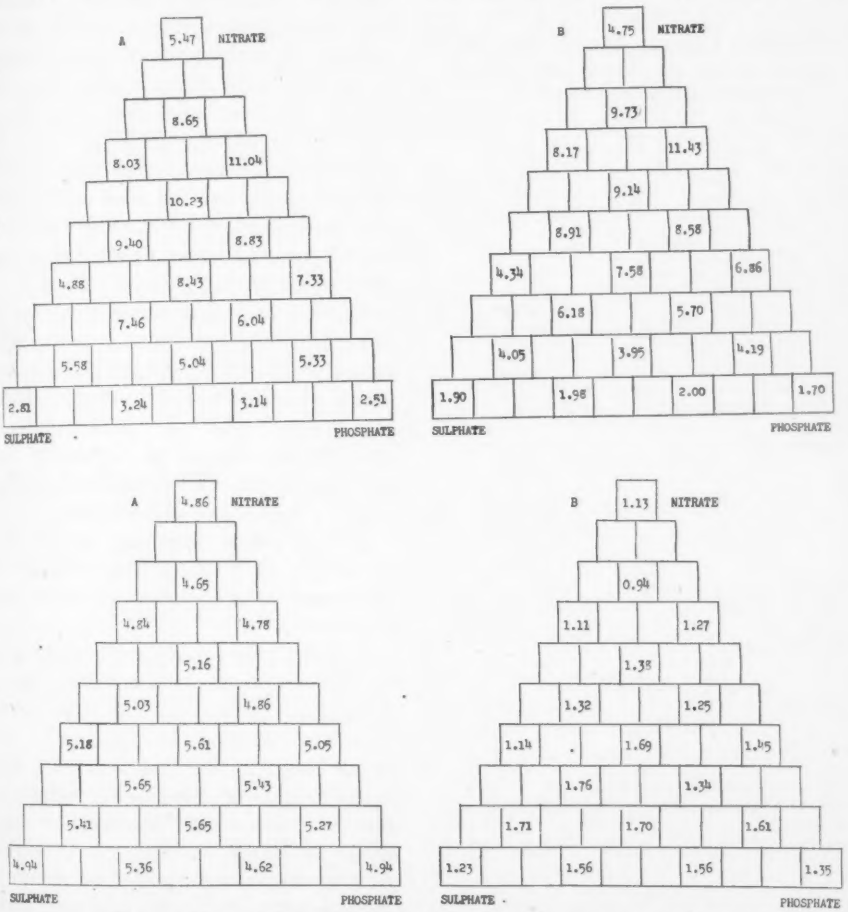

FIG. 1.—*A*, disposition of the 19 nutrient solutions of varying anion composition chosen for test. *B*, same for cation solutions.

used. Figure 3*A* and 3*B* give data on the resin and rubber contents, respectively, of the plants produced in the nineteen anion treatments, while table 3 summarizes the results of the variance analysis. Resin concentration varied but 25% among the several treatments, and the analysis shows that this variation is not significant. The rubber concentrations were all low, as is also true of fieldgrown guayule harvested early in the fall. Still lower rubber concentrations would have been obtained had the plants been harvested 2–3 weeks earlier (about the first of September). Other experiments conducted during the same time and under or of sulphate level on rubber concentration was evident.

In general, treatments tending to produce the largest plants tended also to produce the lowest rubber concentrations. Treatment 2 in particular resulted in more succulent plants, with taller shoots and more impressive bulk, than any other treatment. Treatment 2 also yielded the minimum rubber concentration among the nineteen anion treatments. On the other hand, the smallest plants did not tend to yield the highest concentrations.

Figures 4 and 5 give data on the plants harvested from the nineteen cation

treatments. It is evident that all the responses were smaller than those obtained to varying anion concentrations. Severe deficiencies were not obtained, although there were significant differences in dry weight of stems and roots, dry weight of leaves, and in rubber concentration (table 3). Resin concentration was not significantly affected by the cation treatments. As in the plants of the anion treatments, rubber concentration was low throughout but tended to be somewhat higher in the high than in the low potassium cultures. The lowest rubber concentration occurred in cultures low in both potassium and magnesium but high in calcium.




FIGS. 2, 3.—Harvest of September 15. Fig. 2 (above), dry weight (in grams per two plants produced in the 19 anion treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.34 at 5% level, 1.77 at 1% level; *B*, 1.20 at 5% level, 1.60 at 1% level. Fig. 3 (below), concentration (in percentage dry weight of stems and roots produced in the 19 anion treatments) of *A*, resin; *B*, rubber. Difference needed for significance: *A*, 0.73 at 5% level, 0.97 at 1% level; *B*, 0.40 at 5% level, 0.53 at 1% level.

HARVEST OF MARCH 1.—Experiment I

was continued until March 1, 8.5 months from its initiation and 5.5 months from the first harvest. During the interval between the two harvests, from October through February, the plants of all treatments assumed a dormant aspect. No new shoot growth occurred and no new leaves appeared. The older leaves were shed or knocked off by rain, and total leaf weight decreased. On March 1, new shoot growth began visibly to be initiated, and flower buds were evident on a few plants. Experiments to be reported elsewhere have shown, as noted earlier in this paper, that in the Pasadena area rapid rubber accumulation proceeds through the fall and winter and that the maximum rubber concentration for the season is to be found just at the onset of the spring growing season. Consequently, the rubber concentrations in the plants of experiment I when harvested on March 1 represent the highest values which would be attained in the first season under the conditions used. As with the harvest of September 15, each treatment was represented in the March 1 harvest by sixteen plants; that is, by two plants from each of two cultures from each of the four plots. The plants were dried, weighed, and analyzed as before.

TABLE 3

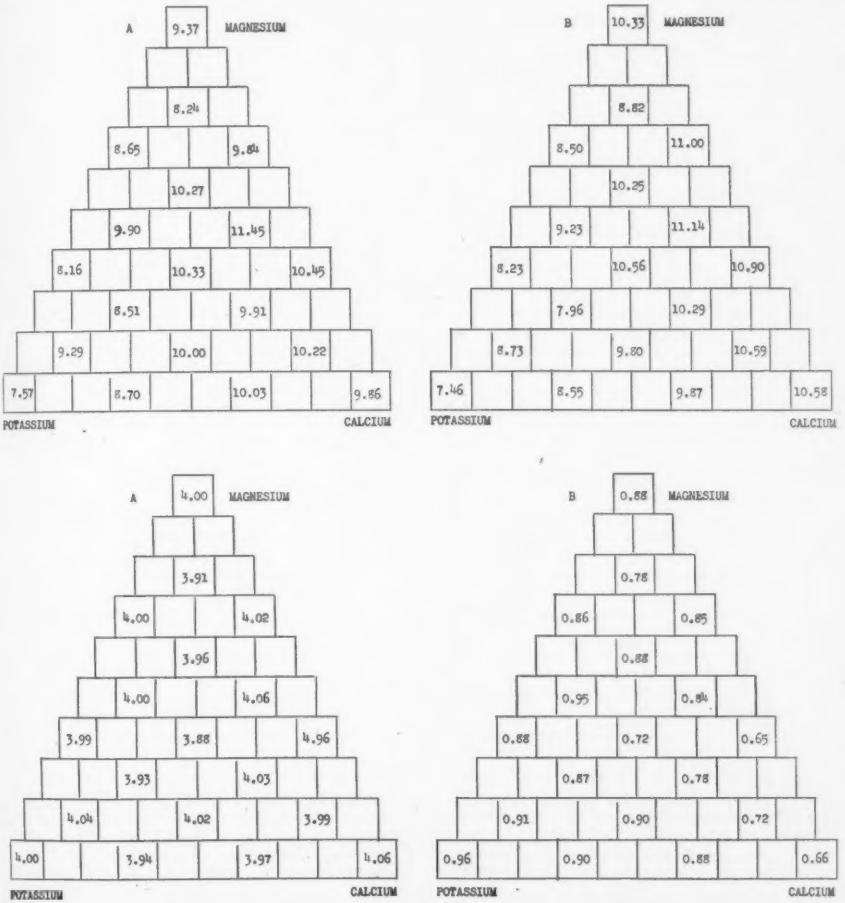
SUMMARY OF F VALUES, DERIVED FROM TREATMENT BY ANALYSIS OF VARIANCE, OF DATA ON GUAYULE PLANTS. EXPERIMENT I

| CHARACTER | F VALUES | |
|---|---|---|
| | Treatments × error | Blocks × error |
| **Anion treatments 1–19; September 15 harvest** | | |
| Dry wt. of stems and roots | 29.90* | 6.00* |
| Dry wt. of leaves | 49.10* | 3.21† |
| Resin (%) | 1.71 | 12.71* |
| Rubber (%) | 3.32* | 2.43 |
| **Cation treatments 21–39; September 15 harvest** | | |
| Dry wt. of stems and roots | 2.18† | 4.70* |
| Dry wt. of leaves | 3.69* | 4.08* |
| Resin (%) | 0.37 | 19.24* |
| Rubber (%) | 4.02* | 7.80* |
| **Anion treatments 1–19; March 1 harvest** | | |
| Dry wt. of stems and roots | 102.65* | 10.48* |
| Dry wt. of leaves | 53.80* | 13.91* |
| Resin (%) | 3.60* | 3.00* |
| Rubber (%) | 37.05* | 0.89 |
| **Cation treatments 21–39; March 1 harvest** | | |
| Dry wt. of stems and roots | 3.48* | 12.81* |
| Dry wt. of leaves | 2.72* | 1.67 |
| Resin (%) | 2.89* | 0.68 |
| Rubber (%) | 3.40* | 6.03* |

* Significant at 1% level. † Significant at 5% level.

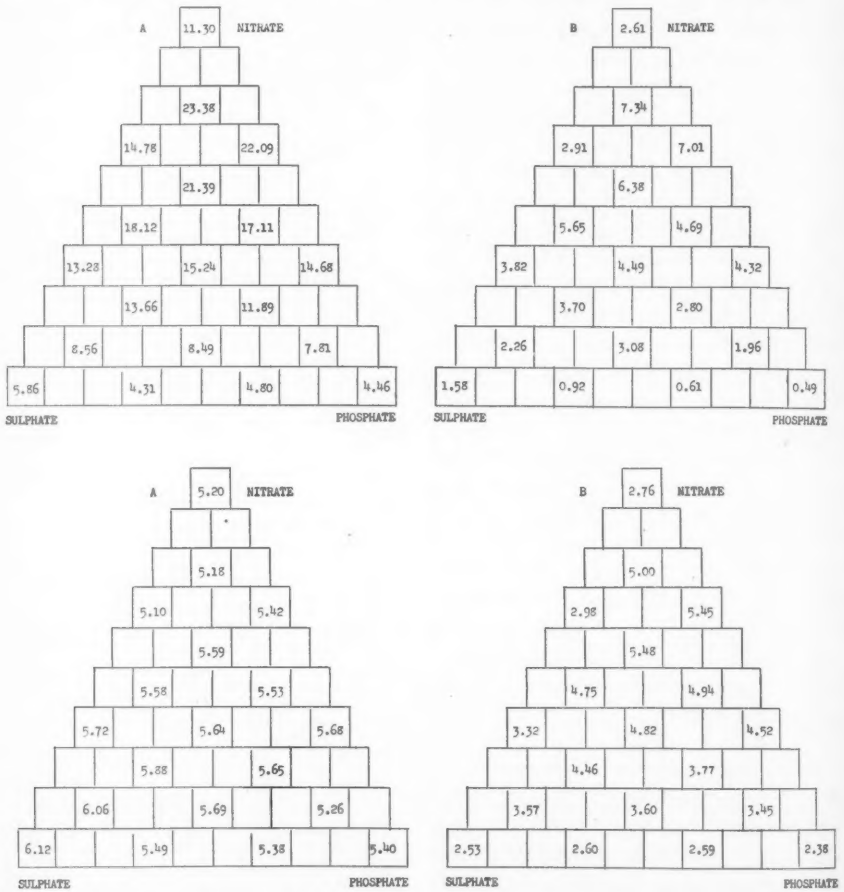
Figure 6 gives data on the stem and root dry weights and the leaf dry weights of the plants grown in the nineteen anion treatments. During the 5.5 months' interval between the two harvests the plants roughly doubled in dry weight of stem and roots but decreased in dry weight of leaves. A winter loss of the older leaves has been observed in other experiments, both in the field and with gravel cultures maintained outdoors. As in the earlier harvest, significant differences in dry weights of stems and roots resulted from the anion treatments (table 3). Nitrogen deficiency produced the smallest plants. Phosphate deficiency appeared only in solution 3 and was not evident at lower nitrogen levels. Sulphate deficiency was not apparent. The largest plants—both as to dry weight of stems

and roots and as to dry weight of leaves—were produced in treatments 2, 4, and 5, which were high in nitrogen and low to moderately high in phosphate but low or very low in sulphate. Figure 7 gives the resin and rubber concentrations found in the plants of the nineteen anion treatments. Significant differences, both in resin and in rubber concentrations, resulted from the varying anion treatments (table 3). As at the September harvest, resin concentration was less influenced than was rubber concentration by the treatments. Thus the largest resin concentration (6.12% in treatment 16) was only 1.2 times as large as the



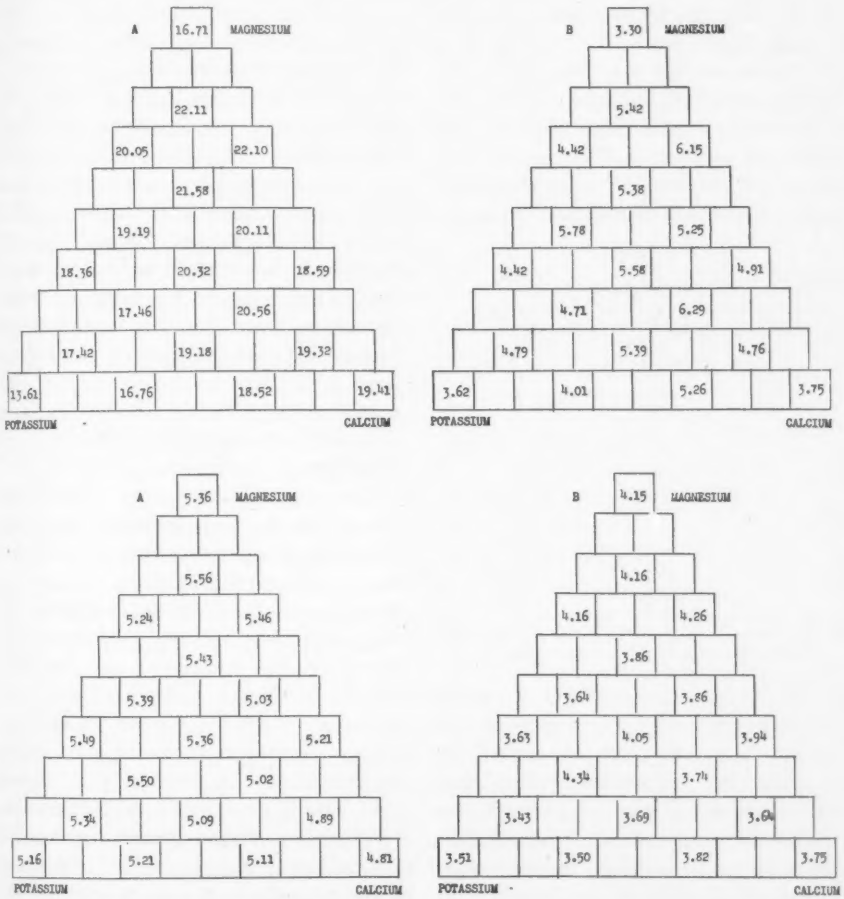

FIGS. 4, 5.—Harvest of September 15. Fig. 4 (above), dry weight (in grams per two plants produced in the 19 cation treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.86 at 5% level, 2.47 at 1% level; *B*, 1.66 at 5% level, 2.21 at 1% level. Fig. 5 (below), concentration (in percentage dry weight of stems and roots produced in the 19 cation treatments) of *A*, resin; *B*, rubber. Difference needed for significance: *A*, 0.36 at 5% level, 0.48 at 1% level; *B*, 0.14 at 5% level, 0.19 at 1% level.

lowest resin concentration (5.10% in treatment 3). The highest rubber concentration (5.48% in treatment 5), however, was 2.3 times as large as the lowest rubber concentration (2.38% in treatment 19). It is also noteworthy that, although rubber concentrations increased markedly during the interval between the two harvests, the resin concentrations increased but little. Comparison of figures 6 and 7 shows that the treatments resulting in the greatest dry weights also resulted in the highest rubber concentrations, while the treatments which resulted in the lowest dry weights resulted in the lowest rubber concentrations. Lower rubber concentration attended both nitrogen and phosphate deficiencies




FIGS. 6, 7.—Harvest of March 1. Fig. 6 (above), dry weight (in grams per two plants produced in the 19 anion treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.67 at 5% level, 2.22 at 1% level; *B*, 0.79 at 5% level, 1.05 at 1% level. Fig. 7 (below), concentration (in percentage dry weight of stems and roots produced in the 19 anion treatments of *A*, resin; *B*, rubber). Difference needed for significance: *A*, 0.41 at 5% level, 0.55 at 1% level; *B*, 0.49 at 5% level, 0.65 at 1% level.

(at high level of nitrogen). The correlation of high rubber concentration with great plant growth is not in agreement with the results of the September 15 harvest, where great plant growth tended to be more nearly inversely related to rubber concentration. Comparison of the results of the September with those of the March harvest shows that rubber concentrations early in the season need not be a reliable index to rubber concentrations of similar plants later in the rubber-accumulating season.




FIGS. 8, 9.—Harvest of March 1. Fig. 8 (above), dry weight (in grams per two plants produced in the 19 cation treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 3.14 at 5% level, 4.12 at 1% level; *B*, 1.42 at 5% level, 1.89 at 1% level. Fig. 9 (below), concentration (in percentage dry weight of stems and roots produced in the 19 cation treatments of *A*, resin; *B*, rubber). Difference needed for significance: *A*, 0.36 at 5% level, 0.47 at 1% level; *B*, 0.41 at 5% level, 0.55 at 1% level.

Figure 8 gives data on the dry weights of plants produced in the nineteen cation treatments. Significant differences in dry

weights of stems and roots, as well as leaves produced, resulted from the treatment (table 3). As in the case of the September harvest, growth responses to the cation deficiencies were less well marked than were the responses to the anion deficiencies. Magnesium deficiency, however, was more severe in the March than in the September harvest. Figure 9 gives data on the resin and rubber concentrations found in the nineteen cation treatments. Significant differences in both quantities resulted from the treatments (table 3), although resin concentration varied by but 16% as between the largest and the smallest values. Rubber concentration was influenced in particular by magnesium concentration, and it decreased regularly as the concentration of this ion in the nutrient decreased. This result is not wholly in accord with those of the September harvest. In the earlier harvest, lowered rubber concentration appeared to result only with nutrients low in both potassium and magnesium, while the highest concentration was found in treatment 16, high in potassium but deficient in both magnesium and calcium. The results of the cation treatments show, as did those of the anion, that treatments advantageous for rubber accumulation early in the season may not maintain their superiority throughout the season.

TABLE 4

IONIC COMPOSITIONS OF SOLUTIONS RESULTING IN MAXIMUM GROWTH AND RUBBER ACCUMULATION

| Solution | Ionic concentration (me./l.) | | | | | |
|---|---|---|---|---|---|---|
| | $NO_3$ | $SO_4$ | $H_2PO_4$ | Mg | K | Ca |
| 2 | 19.8 | 2.8 | 2.8 | 9.0 | 4.5 | 12.0 |
| 4 | 17.0 | 0.0* | 8.5 | 9.0 | 4.5 | 12.0 |
| 5 | 14.2 | 5.7 | 5.7 | 9.0 | 4.5 | 12.0 |
| 22 | 8.8 | 4.7 | 8.8 | 21.0 | 2.0 | 4.0 |
| 24 | 10.0 | 9.0 | 10.0 | 18.0 | 0.0† | 12.0 |
| 25 | 8.7 | 4.8 | 8.7 | 15.0 | 4.0 | 8.0 |

* Maximum of 0.15 me./l. of $SO_4$ present as impurities, etc.
† Maximum of 0.15 me./l. of K present as impurities, etc.

DISCUSSION OF EXPERIMENT 1.—The plants of this experiment, although thrifty and vigorous in appearance, accumulated markedly less dry weight over the 8.5-month experimental period than did similar stock planted in the field. It has been repeatedly observed that guayule plants cultured in 1- to 2-gallon containers fail to make as rapid growth as similar plants in the field. This is not due to deficiency of any major nutrient, as can be seen from the results of the present experiment. Neither does it appear to be due to a deficiency of any micronutrient, since deficiencies for these were not obtainable under the conditions employed.

Maximum growth from a standpoint of dry-weight accumulation was produced in anion treatments 2, 4, and 5 and in cation treatments 22, 24, and 25. In these solutions (table 4) nitrate was present in relatively high concentration (9–20 me./l.). Phosphate was also present in moderate concentrations (2.8–10.0 me./l.), while sulphate varied from 9.0 to 0.15, the latter being the concentration present in solution 4 as impurities. The highest rubber concentrations (5.5%) were obtained in solutions 4 and 5. Rubber accumulation in treatments 4 and 5 compares favorably with the accumulation by fieldgrown plants during the first growing season. It is evident from the compositions of solutions 4 and 5 (table 4) that rubber accumulation—as well as growth—is relatively independent of sulphate concentration but markedly dependent on phosphate and nitrate concentration. Solution 2, which yielded good growth and high rubber concentration, approximates the nutrient

solution of HOAGLAND and ARNON (3) in composition.

Although growth, as measured by dry-weight accumulation, was as great in the optimal solutions of the cation series as in those of the anion series, rubber concentration was markedly less. But the maximum rubber concentrations attained in the cation series do correspond with those in the anion series at comparable nitrate levels. In a repetition of experiment I, it would be desirable to use the optimum anion concentrations in the composition of the cation solutions. Conversely, however, the concentrations of cations used in the composition of the anion solutions of experiment I would appear to have been near optimal. It should be noted that although treatment 9 is identical with treatment 29, still there are significant differences in growth and rubber concentrations as between plants grown under the two treatments. This reflects the fact already mentioned that the cation and anion treatments were set up as separate experiments in separate plots.

### Experiment II

During the second week in June, 1942, three nursery seedlings of the same lot as used in experiment I were planted in each of approximately 200 1-gallon cans. The cans, which had been previously painted with nontoxic asphaltum paint and provided with drainage, were filled with 4-mesh gravel. On July 1, the plants were thinned to two per container, and 140 cultures selected for uniformity were chosen for the initiation of experiment II.

NUTRIENT SOLUTIONS.—The seven nutrient solutions used in experiment II supplied nitrogen either entirely as nitrate, entirely as ammonium, or as mixtures of the two. The solutions (table 5) were modified from those suggested by VICKERY *et al.* (4). K, $PO_4$, Ca, N, and Mg are present in the same concentrations in all seven solutions (table 7). Both Cl and $SO_4$ increase as the ratio of ammonium to nitrate increases. Total osmotic pressure of the ammonium-containing solutions is likewise greater than that of the solution containing only nitrate nitrogen. A preliminary experiment indicated that, over a 3-month growth period, variations in osmotic pressure of the nutrient between the limits of the solutions of table 7 exert only minor effects on growth. VICKERY *et al.* have also shown for tobacco that the influence of the added Cl and $SO_4$ concentrations is negligible in comparison with the influence of the ammonium ion itself.

TABLE 5

CONCENTRATIONS OF INGREDIENTS OF NUTRIENT SOLUTIONS SUPPLYING NITROGEN AS NITRATE AND AMMONIUM AT DIFFERENT RATIOS (MILLIMOLS PER LITER)

| SALT | SOLUTION NUMBER | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| | Percentage N as $NO_3$ | | | | | | |
| | 100 | 80 | 60 | 40 | 20 | 10 | 0 |
| $KH_2PO_4$ | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 |
| $Ca(NO_3)_2$ | 8.6 | 6.9 | 5.2 | 3.4 | 1.7 | 0.9 | 0.0 |
| $CaCl_2$ | 0.0 | 1.7 | 3.4 | 5.2 | 6.9 | 7.7 | 8.6 |
| $(NH_4)_2SO_4$ | 0.0 | 1.7 | 3.4 | 5.2 | 6.9 | 7.7 | 8.6 |
| $MgSO_4$ | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 | 2.1 |

Nutrient solution was applied at the rate of 300 cc. per culture three times a week. Tap water was further supplied as needed. All nutrient solutions were adjusted to pH 5.6 before use.

EXPERIMENTAL DESIGN.— The seven treatments of experiment II were tested in each of five plots. In each plot each treatment was represented by four cul-

tures, each of two plants. Thus each treatment was tested on forty plants. The data were treated by the analysis of variance.

HARVEST OF OCTOBER 1.—On October 1, after 3 months of treatment, twenty plants of each treatment were harvested, representing two plants in each of two cultures from each of the five plots. The plants were washed free of sand, dried rapidly at 60° C., weighed (the leaves separately), and the stem and root portions ground in a Wiley mill and analyzed for rubber and resin.

TABLE 6

INFLUENCE OF NUTRITION WITH VARYING RATIOS OF AMMONIUM TO NITRATE NITROGEN ON GROWTH AND RUBBER ACCUMULATION. HARVEST OF OCTOBER 1

| TREATMENT NO. | $NO_3$ (%) | $NH_4$ (%) | DRY WT. PER 2 PLANTS (GM.) | | RESIN‡ (%) | RUBBER§ (%) |
|---|---|---|---|---|---|---|
| | | | Stems and roots* | Leaves† | | |
| 1 | 100 | 0 | 10.21 | 11.28 | 3.83 | 1.28 |
| 2 | 80 | 20 | 11.20 | 12.13 | 3.78 | 1.16 |
| 3 | 60 | 40 | 11.73 | 12.16 | 3.92 | 1.14 |
| 4 | 40 | 60 | 10.90 | 11.41 | 4.07 | 1.16 |
| 5 | 20 | 80 | 9.81 | 10.31 | 4.32 | 1.14 |
| 6 | 10 | 90 | 7.86 | 8.77 | 4.00 | 1.07 |
| 7 | 0 | 100 | 6.83 | 8.15 | 4.00 | 0.76 |

* Difference of 1.49 required for significance at 5% level; of 1.98 at 1% level.

† Difference of 1.61 required for significance at 5% level; of 2.14 at 1% level.

‡ Difference of 0.21 required for significance at 5% level; of 0.28 at 1% level.

§ Difference of 0.25 required for significance at 5% level; of 0.33 at 1% level.

Table 6 summarizes the values obtained for dry weight, percentage resin, and percentage rubber. Plants receiving 0, 20, 40, 60, or 80% of their nitrogen as ammonium did not differ significantly as to dry weight of stem and root, dry weight of leaves, or percentage of rubber, while percentage of resin was but slightly affected. Plants which received 90% of their nitrogen as ammonium, however, were markedly reduced in dry weight and in rubber concentration as compared with plants receiving less ammonium ion. Still more marked reduction in dry weight and rubber concentration resulted from the application of 100% of the nitrogen as ammonium.

HARVEST OF MARCH 1.—During the late fall and winter months, the plants of all treatments maintained a dormant aspect. On March 1, after 8 months of treatment, the second harvest was made. Each treatment was again represented by two plants from each of two cultures from each of five plots, a total of twenty plants. The plants were dried, weighed, and analyzed as before. Table 7 gives a summary of the data. In experiment II, as in experiment I, marked increases in dry weight of stems and roots took place during the winter, while dry weight of leaves decreased owing to shedding of the older leaves. Treatments in which 0, 20, 40, or 60% of the nitrogen was present as ammonium did not result in plants which differed significantly from one another as to dry weight of stems and roots, percentage of resin, or percentage of rubber. The plants which received 80, 90, or 100% of their nitrogen as ammonium yielded progressively lower dry weights and rubber concentrations.

DISCUSSION OF EXPERIMENT II.—The results of experiment II parallel those of CLARK (1) with tomato and of VICKERY (4) with tobacco, in that growth as measured by accumulation of dry weight was decreased as the ratio of ammonium nitrogen to nitrate nitrogen in the medium was increased. Both CLARK and VICKERY have pointed out the great influence which the type of the available nitrogen exerts on the composition of the

plant. In the present experiment a significant reduction in rubber concentration was apparently brought about as a result of the presence of considerable amounts of ammonium ion in the nutrient solution, pointing again to the importance of the kind of available nitrogen in determining plant constitution. Significant reduction in rubber percentage, in both the October and the March harvests, resulted exclusively in treatments where dry weight accumulation was likewise significantly reduced as a result of high ammonium nitrate ratios. While the physiological significance of the apparent deleterious effect of ammonium ions on growth and rubber accumulation is not evident at present, still this experiment does indicate that the use of ammonium ions in the nutrition of guayule plants may be undesirable under some conditions.

TABLE 7

INFLUENCE OF NUTRITION WITH VARYING RATIOS OF AMMONIUM TO NITRATE NITROGEN ON GROWTH AND RUBBER ACCUMULATION. HARVEST OF MARCH 1

| Treatment no. | $NO_3$ (%) | $NH_4$ (%) | Dry wt. per 2 plants (gm.) | | Resin‡ (%) | Rubber§ (%) |
|---|---|---|---|---|---|---|
| | | | Stems and roots* | Leaves† | | |
| 1 | 100 | 0 | 17.71 | 5.98 | 4.54 | 4.46 |
| 2 | 80 | 20 | 18.75 | 6.78 | 4.78 | 4.64 |
| 3 | 60 | 40 | 17.42 | 5.35 | 4.56 | 4.37 |
| 4 | 40 | 60 | 18.54 | 4.29 | 4.64 | 4.62 |
| 5 | 20 | 80 | 14.95 | 2.77 | 4.49 | 4.36 |
| 6 | 10 | 90 | 12.71 | 2.69 | 4.42 | 3.24 |
| 7 | 0 | 100 | 10.83 | 1.66 | 4.08 | 2.51 |

* Difference of 3.11 required for significance at 5% level; of 4.13 at 1% level.

† Difference of 0.98 required for significance at 5% level; of 1.30 at 1% level.

‡ Difference of 0.24 required for significance at 5% level; of 0.32 at 1% level.

§ Difference of 0.51 required for significance at 5% level; of 0.68 at 1% level.


## Summary

1. Guayule plants were grown outdoors in gravel culture over a period of 8.5 months and supplied with thirty-eight different nutrient solutions of varying anion and cation composition. Two harvests were made, one at the end of the summer growing season and one at the end of the winter—or principal rubber-accumulating season.

2. Growth and rubber accumulation were affected by nitrogen supply more than by the supply of any other major nutritional element under the conditions used. Plants which received little nitrogen and which showed reduced growth accumulated little rubber as compared with plants at higher nitrogen levels. The highest level of rubber accumulation was in plants which received 14–17 milliequivalents of nitrogen as nitrate per liter of nutrient solution. These plants contained 5.5 % rubber, based on dry weight of the defoliated plant, at the winter harvest.

3. Growth and rubber accumulation were both diminished in phosphate-deficient plants as compared with plants which received abundant phosphate. No responses to sulphate deficiency were evident.

4. Growth and rubber accumulation were relatively independent of the concentrations of calcium and potassium in the nutrient solution, but both were depressed under conditions of low magnesium concentration.

5. Plants were supplied with nitrogen in the form of nitrate or of ammonium, or as mixtures of the two. Those which received most or all of their nitrogen as ammonium grew less and accumulated less rubber over an 8-month period than plants which received all or most of their nitrogen as nitrate.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LITERATURE CITED

1. CLARK, H. E., Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. 11:5–24. 1936.
2. HAMNER, C. L., Growth responses of Biloxi soybeans to variation in relative concentrations of phosphate and nitrate in the nutrient solution. BOT. GAZ. 101:637–649. 1940.
3. HOAGLAND, D. R., and ARNON, D. I., The water culture method of growing plants without soil. Univ. Calif. Coll. Agr. Circ. 347. 1938.
4. VICKERY, W. B., PUCHER, G. W., WAKEMAN, A. S., and LEAVENWORTH, C. S., Chemical investigations of the tobacco plant. VIII. The effect on the composition of the plant of the form in which nitrogen is supplied. Conn. Agr. Exp. Sta. Bull. 442. 1940.

# EXTENT OF AUXIN-PRECURSOR HYDROLYSIS IN DIFFERENT AVENA ASSAY METHODS

G. S. AVERY, JR., J. BERGER, AND B. SHALUCHA

## Introduction

In a recent study of auxins (10) the statement was made that the "deseeded test responds not only to auxin but also to auxin precursor."[1] It was obviously important to test the validity of this view, as applied to both the deseeded and modified standard methods, since the deseeded *Avena* test has been used in this laboratory for the past 4 years. Our study shows that in neither test method is there a significant conversion of precursor to auxin.

When the deseeded test method was first described (9), it was pointed out that it was designed for the detection of precursors of auxin. SKOOG, however, showed that no curvatures were obtained from *Avena* auxin precursor in the deseeded test in 8 hours; after 15 hours he reported curvatures. WENT (10) concluded that the response of deseeded plants to auxin diffused from freshly excised *Avena* coleoptile segments is almost exclusively due to auxin precursor. If the duration of the test was less than 10 hours (and the actual time is not specified in the paper in question, although a private communication from Professor WENT states that the time was 5 hours), then this is in direct contradiction to SKOOG's findings. We wish to emphasize at this point that the deseeded test as used in our laboratory is of exactly 5 hours' duration; that is, from time of application of agar blocks to the time of photographing.

MATERIAL AND METHODS.—Three general types of material were used as sources of auxin precursor: (*a*) extracts of immature and mature (dormant) kernels of *Zea mays* prepared in various ways; (*b*) a zein preparation from maize kernels; and (*c*) purified auxin-precursor preparations from maize kernels. These materials were extracted or otherwise treated as indicated in the tables and in most instances were assayed by both the deseeded (1) and the modified standard (8) *Avena* test methods. All data are based on *Avena* curvatures in the proportionality range.

## Investigation

When WENT's statement appeared to the effect that "the curvature in the deseeded test is almost exclusively due to auxin precursor . . . ." with certain auxin preparations, we reviewed some of the data then on hand from work in our

[1] The term "precursor" as used here indicates a compound which is physiologically inactive in the *Avena* test but becomes auxin (physiologically active) upon hydrolysis.

laboratory. These are presented in table 1. The examples were selected because of their low "free" auxin content and high precursor yield, as determined by auxin activity after alkaline hydrolysis. Even if it is assumed that the entire yields in untreated extracts represent precursor converted to auxin by deseeded plants during the 5-hour test, then 1 or 2% is the maximum possible conversion.

Further to test whether the response to unhydrolyzed maize extracts was due to free auxin or to auxin converted from the precursor during the test, aqueous extracts were purified by ether extraction to remove as much of the free auxin as possible. Aqueous extracts of ground whole kernels of sugar corn were made at natural pH, then adjusted to pH 4–5 and shaken with three changes of ethyl ether (25 ml. each). The ether extracts containing the auxin were discarded. The assays given in table 2 are on the water residue, which is high in precursor. Part of the residue was assayed without further treatment, and part after alkaline hydrolysis; that is, autoclaved 30 minutes in M/20 borate buffer, pH 9.6, then adjusted to pH 6 before assay. Results of assays on such purified extracts are given in table 2. The important fact to note is that in spite of the ether purification, 0.7–1.9% of the total auxin activity still appears as "free" auxin. The question whether this figure represents residual traces of free auxin, or small amounts of auxin produced by actual conversion of precursor during the assay, cannot be answered by these data; they justify no more than a repetition of the first observation that if conversion does occur, it is less than 1–2%.

Conclusive evidence could be obtained only from purer preparations of precursor. Data on such preparations are given in table 3. The zein sample was one that had been thoroughly extracted with ether; the precursor samples were

TABLE 1

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING DESEEDED AVENA TEST, AS SHOWN BY ASSAYS ON AQUEOUS EXTRACTS OF GROUND CORN KERNELS. DATA FOR SAMPLES 1 AND 5 PUBLISHED PREVIOUSLY (4); PEDIGREES, ETC., AS IN EARLIER PAPER

| Sample no. | Material | Yield of auxin, in millions of TDC* or tens of micrograms of indoleacetic acid (per gram dry weight) of corn | | Maximum possible precursor (%) converted to auxin during test |
|---|---|---|---|---|
| | | Untreated† | Alkali hydrolyzed‡ | |
| 1.... | Inbred 77, 10 days after pollination | 0.80 | 34.7 | 2.3 |
| 2.... | Hybrid 677×C14 ($F_2$), 12 days after pollination | 0.30 | 23.4 | 1.3 |
| 3.... | Inbred 75, 20 days after pollination | 1.60 | 92.8 | 1.7 |
| 4.... | Inbred 580, 58 days after pollination | 0.20 | 19.7 | 1.0 |
| 5.... | Country Gentleman, mature kernels | 0.20 | 10.0 | 2.0 |
| 6.... | Hybrid 75×77, mature kernels | 0.39 | 18.9 | 2.1 |

*TDC (total degrees curvature), usual units employed in this laboratory (2), are defined in terms of deseeded test and applicable only to it.

† Ground tissue extracted with water at about 25° C. for 15 minutes; suspensions centrifuged and clear centrifugate assayed for auxin activity.

‡ Ground tissue heated in M/20 borate buffer, pH 9.6, at 120° C. for 15 minutes; suspensions then adjusted to pH 6 and clear centrifugates assayed for auxin.

isolated from ground corn kernels in a manner described elsewhere (5). After alkaline conversion, preparation DE-5—for example—was 2.4% pure indoleacetic acid, calculated on a dry-weight basis. It may be seen from the data that with the zein, a maximum of 0.35%

TABLE 2

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING AVENA ASSAY AS SHOWN BY ASSAYS ON PARTIALLY PURIFIED AQUEOUS EXTRACTS OF SWEET CORN

| SAMPLE NO. | MATERIAL | TREATMENT OF ETHER-EXTRACTED WATER RESIDUE | AVENA ASSAYS* | | | | MAXIMUM† POSSIBLE PRECURSOR (%) CONVERTED TO AUXIN DURING TEST | |
|---|---|---|---|---|---|---|---|---|
| | | | Deseeded | | Modified standard | | | |
| | | | Dilution (ml.) | Curvature (°) | Dilution (ml.) | Curvature (°) | Deseeded | Modified standard |
| 1..... | Frozen sweet corn (Golden Bantam) | None | 100 | 13.9 | 25 | 9.0 | ..... | ..... |
| | | Alkali hydrolyzed | 8000 | 9.1 | 4000 | 8.2 | 1.9 | 0.7 |
| 2..... | Frozen sweet corn (Golden Bantam) | None | 200 | 10.2 | 25 | 9.7 | ..... | ..... |
| | | Alkali hydrolyzed | 8000 | 12.9 | 4000 | 9.1 | 2.0 | 0.7 |
| 3..... | Fresh corn (Early Yellow) | None | 200 | 9.4 | 50 | 10.8 | ..... | ..... |
| | | Alkali hydrolyzed | 8000 | 9.1 | 4000 | 10.5 | 2.6 | 1.3 |
| 4..... | Fresh corn (Early Yellow) | None | 200 | 13.1 | 50 | 20.8 | ..... | ..... |
| | | Alkali hydrolyzed | 8000 | 11.7 | 8000 | 7.1 | 2.8 | 1.8 |

* Controls as follows: Deseeded test, 10 micrograms indoleacetic acid per liter, 9.2° curvature; modified standard test, 20 micrograms indoleacetic acid per liter, 8.2° curvature.

† Maximum percentage hydrolysis: Assuming that all the yield in the untreated preparation is due to hydrolysis of the precursor by the *Avena* coleoptile, then, for sample no. 1, deseeded method, the conversion is $\frac{13.9}{9.1} \times \frac{100}{8000} \times 100 = 1.9\ \%$.

TABLE 3

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING AVENA TEST AS SHOWN BY ASSAYS ON ZEIN AND PURIFIED CORN AUXIN PRECURSOR

| SAMPLE | TREATMENT | AVENA ASSAYS | | | | PRECURSOR (%) APPARENTLY CONVERTED TO AUXIN DURING TEST | |
|---|---|---|---|---|---|---|---|
| | | Deseeded | | Modified standard | | | |
| | | Dilution‡ (ml.) | Curvature in degrees | Dilution‡ (ml.) | Curvature in degrees | Deseeded | Modified standard |
| Zein* | None | 75 | 10.9 | 37.5 | 7.5 | ..... | ..... |
| | Alkali hydrolyzed | 30,000 | 7.7 | 15,000 | 8.5 | 0.35 | 0.22 |
| Purified auxin precursor DE-5† | None | 250 | 13.4 | 125 | 10.0 | ..... | ..... |
| | Alkali hydrolyzed | 1,600,000 | 14.8 | 400,000 | 17.5 | 0.014 | 0.018 |

* Zein preparation extracted for 3 days in ether by Dr. S. A. GORDON, to whom we are indebted for the material.

† Precursor preparation of 2.4% purity.

‡ Calculated to basis of 1 gm. of sample tested.

conversion was obtained in the deseeded assay, as against 0.22% in the modified standard test. With precursor DE-5 the extremely small "apparent" conversion value of 0.014% was obtained in the deseeded test. Approximately the same value was obtained in the modified standard test. Other precursor concentrates gave apparent conversions of equal magnitude in both tests. With increasing purification of precursor, the conversion value decreased. Thus it is highly probable that even the small curvatures produced by unhydrolyzed precursor concentrates are due to contaminating amounts of free auxin and not to precursor conversion at all. Precursor preparation DE-5 is typical of several isolations made from corn. Since these purified concentrates contain as high as 50% of the precursor present in corn, it may be assumed that similar resistance to conversion exists for the major portion of the auxin precursor in corn. In the light of these results, the apparent conversion values in tables 1 and 2 may be interpreted as representing free auxin and not real conversion.

It is obvious that if there is any conversion of maize auxin precursor to auxin in either test method, it is so small as to be of no consequence in assays of natural extracts.

## Discussion

The evidence presented here supports a portion of SKOOG's work and may be briefly summarized as follows: curvatures obtained during the 5-hour test period from untreated aqueous extracts of corn represent free auxin, and not auxin converted from a precursor. The evidence presented is equally applicable, whether maize extracts contain one or more than one auxin precursor.

As to the non-hydrolyzability during the deseeded test of auxin precursors other than that from corn, it may be suggested that auxin precursors from all the cereal grains will probably respond in a similar manner. Precursors have been demonstrated by the deseeded method in maize, wheat, oats, rice, and even in turnip and beet seeds by assays on untreated and alkali hydrolyzed preparations (2). The detection of precursors in these materials rests clearly on alkaline hydrolysis of the preparation before assay and not upon conversion by the test plant.

The response of deseeded test plants to a variety of synthetic compounds of a precursor nature has already been studied: naphthalene and indole acetamides, methyl naphthaleneacetate (3), indole ethylamine and tryptophan (9), all show no activity in a 5-hour deseeded test. After chemical hydrolysis, however, these compounds produce *Avena* curvatures.

It seems more or less unfortunate that the impression has crept into the literature (10, 11) that deseeded test plants respond to auxin precursor in 2–6 hours. This is not in agreement with the experimental data presented by SKOOG, although it is apparently upon his work that the impression is based. Figure 29 in (11) contains no curve to substantiate the statement in the text that agar blocks containing auxin precursor, if left on the deseeded test plants 2–6 hours, give rise to distinct auxin curvatures. SKOOG's original curve (9, curve 2, fig. 8) shows no measurable curvature from precursor until 15 hours.

In our experience the same figure 29 (11) gives a misleading impression as regards the response of deseeded test plants to pure indoleacetic acid. The response in the 5-hour deseeded test is strictly a proportional one (as related to time and concentration) when the concentration

is between 0 and 20 micrograms per liter (fig. 1). The odd shape of curve A in figure 29 (11) is difficult to understand, since a 100-microgram per liter solution of indoleacetic acid gives the response shown in figure 1 of this paper; our test even at this relatively high concentration also shows proportional response up to

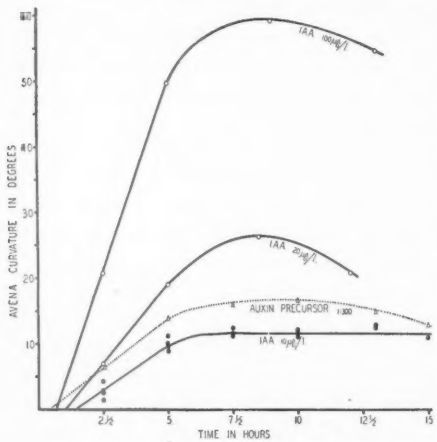


FIG. 1.—Coleoptile curvatures in *Avena* test plants over 15-hour test period (deseeded method). Indoleacetic acid (IAA) in 1.5% agar, tested at concentrations of 10, 20, and 100 micrograms per liter; partially purified maize auxin precursor (5) tested at 1:300 dilution. For IAA curves, each point is average of 2 dozen test plants (one day's run); for auxin precursor preparation, each point on curve is average of 4 dozen test plants (one day's run). Darkroom maintained at 88–90% relative humidity, 25° C., and lighted only with phototropically inactive light.

5 hours. (We prefer to work with curvatures of less than 20 degrees.)

The slope of the curve for the auxin precursor is in close agreement with those produced by 10 micrograms per liter solutions of indoleacetic acid (fig. 1); the *Avena* curvatures are undoubtedly due to a small amount of free auxin in the precursor preparation. This fact can be regarded only as supporting the other evidence as to lack of precursor conversion in the deseeded test plant. If there were conversion of the precursor into auxin, a steeper slope would be expected in the curve as well as a continuing rise after $7\frac{1}{2}$ hours.

Does the deseeded test plant indeed convert auxin precursor(s) into auxin, even after 15 hours? In the light of the work by GORDON and WILDMAN (6), who show that a small amount of auxin is produced when tryptophan is heated gently (65° C.) for 1 minute with agar, part of SKOOG's original experiments might be explained as follows: In the preparation of the tryptophan in agar blocks, the short period of heating resulted in the formation of a very small amount of auxin, which, over a long period of time, gave curvatures of small magnitude (SKOOG reported approximately 5 degrees). Or, if there was no conversion of tryptophan into auxin during the preparation of agar blocks, it is equally possible that such a conversion occurred in the blocks at darkroom temperatures in the course of the 15-hour period they were on the test plants. In neither case would the curvatures be the result of precursor entering the test plants as such, and there being converted into auxin.

The curvature obtained with *Avena* precursor extracts might similarly have been due to a response of the deseeded plant to a very small amount of free auxin, and not to precursor conversion. The fact that the deseeded test continues for 5 hours enables it to detect much smaller amounts of auxin than are detectable by the original or modified standard methods ($1\frac{1}{2}$ hours).

Bearing indirectly on this problem is VAN OVERBEEK's (7) work on "exhaustive diffusion" and "exhaustive extractions," from which he concludes that decapitated coleoptile tips (of maize and oats) standing on agar for periods of

many hours slowly convert precursor into auxin, and in the latter form deliver it to the agar by diffusion. This apparently means that excised coleoptile tissues can convert precursor into auxin; it does not indicate that the test plant is in any way concerned in converting precursor to auxin.

There is as yet no certain evidence that the coleoptiles of test plants respond to auxin precursor as such, in agar, and convert it into auxin, ultimately producing curvature in the *Avena* test (whether it be the deseeded test or some other).

**Summary**

1. It has been stated in the literature that deseeded *Avena* test plants respond to auxin precursor in 2–6 hours. The evidence presented here, as a result of studies on crude and purified preparations of a maize auxin precursor, shows that curvatures obtained during the 5-hour test period represent free auxin, and not auxin converted from a precursor.

2. The question is raised whether under any conditions deseeded test plants ever convert precursor into auxin, even if employed for long test periods.

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LITERATURE CITED

1. AVERY, G. S., JR., CREIGHTON, H. B., and HOCK, C., A low cost chamber for phytohormone tests. Amer. Jour. Bot. **26**:360–365. 1939.
2. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., The total extraction of free auxin and auxin precursor from plant tissue. Amer. Jour. Bot. **28**:596–607. 1941.
3. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., Comparative activity of synthetic auxins and derivatives. BOT. GAZ. **104**:281–287. 1942.
4. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., Auxin content of maize kernels during ontogeny, from plants of varying heterotic vigor. Amer. Jour. Bot. **29**:765–772. 1942.
5. BERGER, J., and AVERY, G. S., JR., Isolation of an auxin precursor and an auxin (indoleacetic acid) from maize. Amer. Jour. Bot. (in press) 1944.
6. GORDON, S. A., and WILDMAN, S. G., The conversion of tryptophane to a plant growth substance by conditions of mild alkalinity. Jour. Biol. Chem. **147**:389–398. 1943.
7. OVERBEEK, J. VAN, A quantitative study of auxin and its precursor in coleoptiles. Amer. Jour. Bot. **28**:1–10. 1941.
8. SCHNEIDER, C. L., and WENT, F. W., A photokymograph for the analysis of the *Avena* test. BOT. GAZ. **99**:470–496. 1938.
9. SKOOG, F., A deseeded *Avena* test method for small amounts of auxin and auxin precursors. Jour. Gen. Physiol. **20**:311–334. 1937.
10. WENT, F. W., Growth, auxin, and tropisms in decapitated *Avena* coleoptiles. Plant Physiol. **17**:236–249. 1942.
11. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

## APOSPORY AND APOGAMY IN A SPECIES OF TECTARIA

W. N. STEIL

Spores of a *Tectaria* species, labeled *Dryopteris trifoliata* but undoubtedly *Tectaria trifoliata*, obtained from the Botanical Garden of Leiden, Holland, April, 1935, were sown on the surface of a Beyernick's solution, as modified by MOORE (5), and which had first been put into Erlenmeyer flasks and sterilized in an autoclave. The observations on the gametophyte of this fern in the first cultures extended over a period of a year and were confirmed by two other sets of cultures, the spores of which were later obtained from the same source and sown September, 1935, and June, 1939.

### Observations

GAMETOPHYTE.—In most respects the gametophyte resembles that of a *Dryopteris* species in its heart-shaped form and

large size. There is a tendency to lobing, however, even when it is grown under favorable light conditions. The hairs, so characteristic of the gametophyte of *Dryopteris*, are absent. Archegonia are never produced, but antheridia with normal antherozoids are formed in considerable numbers.

FIGS. 1, 2.—*Tectaria* species: Fig. 1, variegated gametophyte, shaded portions representing darker chlorophyllose regions. Tonguelike outgrowth with tracheids (*t*) bears young apogamous embryo. Fig. 2, apogamously produced sporophyte with gametophytes of aposporous origin.

The majority of the gametophytes were characterized by peculiar regions, varying from nearly colorless to dark green cells. The cells of the nearly white portions contained fewer and paler plastids, some of them being colorless. The thickness of the walls of the cells varied with their color, the greenest ones possessing the thickest walls. Frequently the nearly albinic cells were considerably smaller than the ordinary chlorophyllose cells. A large gametophyte with four dark green regions is shown in figure 1. Such regions were also observed in the gametophytes of aposporous origin. Similar gametophytes of several other *Tectaria* species and of a *Dryopteris* species have also been observed. Earlier, fern gametophytes of this nature have been described by ANDERSSON (1) and ANDERSSON-KOTTÖ (2, 3).

APOGAMOUS EMBRYO.—The embryo is of apogamous origin. The first evidence of such an embryo, as has been found in other apogamous ferns (4, 6, 7, 9, 10), is the appearance of tracheids in a less chlorophyllose region just back of the apical notch of the gametophyte.

In the sinus of the prothallium a lobe is always produced, from which a flat, tonguelike, cylindrical, or conical process may be formed and in which tracheids may also appear (fig. 1). On some portions of the projection the apogamous sporophyte originates. The embryo develops in the manner first described by FARLOW (4) in *Pteris cretica* var. *albolineata*, namely, in order of parts: leaf, root, and stem (the foot being absent).

At the base of the embryo (fig. 1), and from the petiole and blade of the young sporophyte, hairs—each consisting of several cells—are produced in large numbers. The hair possesses a sharp-pointed terminal cell (fig. 4) which bears fewer chloroplasts than the others, and hence is often almost colorless.

APOSPOROUS GAMETOPHYTE.—This originates in several ways (figs. 2–4), the

most common one being from one or more cells of a hair of the apogamously produced sporophyte. The nearly colorless terminal hair cell is apparently never involved in the formation of a gametophyte.

The first visible evidence of the origin of a gametophyte from a hair is the multiplication of the number of chloroplasts in its cells (figs. 4, 5). The gametophytes of this nature, originating from the hairs at the base of the embryo, are produced in great numbers, and sometimes several are formed from the hairs of a single petiole of the first leaf (fig. 6). No gametophytes were observed to develop from the hairs of the blade of the leaf of the sporophyte.

The gametophyte originates from any cells of the hair except the terminal one (figs. 4–9), as already stated. Only once was a rhizoid observed to be formed (fig. 8) from a gametophyte of aposporous origin. Only early stages in the development of the gametophyte were studied, but it can be assumed that the later stages are in all respects similar to those originating from a spore.

Of less frequent occurrence are the gametophytes originating either from marginal epidermal cells or from epidermal cells near the margin of the first leaf of the sporophyte (fig. 2). As is shown in the figure, a considerable number of gametophytes of this nature may be produced.

The most interesting origin of the gametophyte is illustrated in figure 3, showing the terminal portion of the leaf blade. A lobe, similar to that shown at the left, was also present at the right but was injured in mounting. A large part of the end of the leaf is distinctly gametophytic. This portion has produced plates of cells which in turn formed smaller plates and filaments. The conspicuous veins extend into regions distinctly gametophytic.

A number of "lighter" regions, including some which are albinic, can be observed in different portions of the leaf as well as in the terminal gametophytic outgrowths. A small terminal portion of another similar leaf is shown in figure 10.

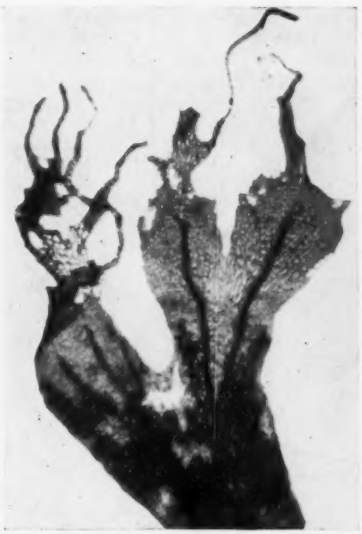

FIG. 3.—Aposporous gametophytes produced by first leaf of sporophyte of apogamous origin.

The longer narrower cells represent those near the sinus of the modified leaf. The lighter areas could be readily observed with the low power of the microscope.

The writer (**8, 10**) has suggested the nature of the origin of apospory of the type just described by assuming, on account of the intimate connection between the sporophyte of apogamous origin and its gametophyte, that some of the cells may be carried upward by the developing leaf and, retaining their power to divide, may form ordinary gametophytes.

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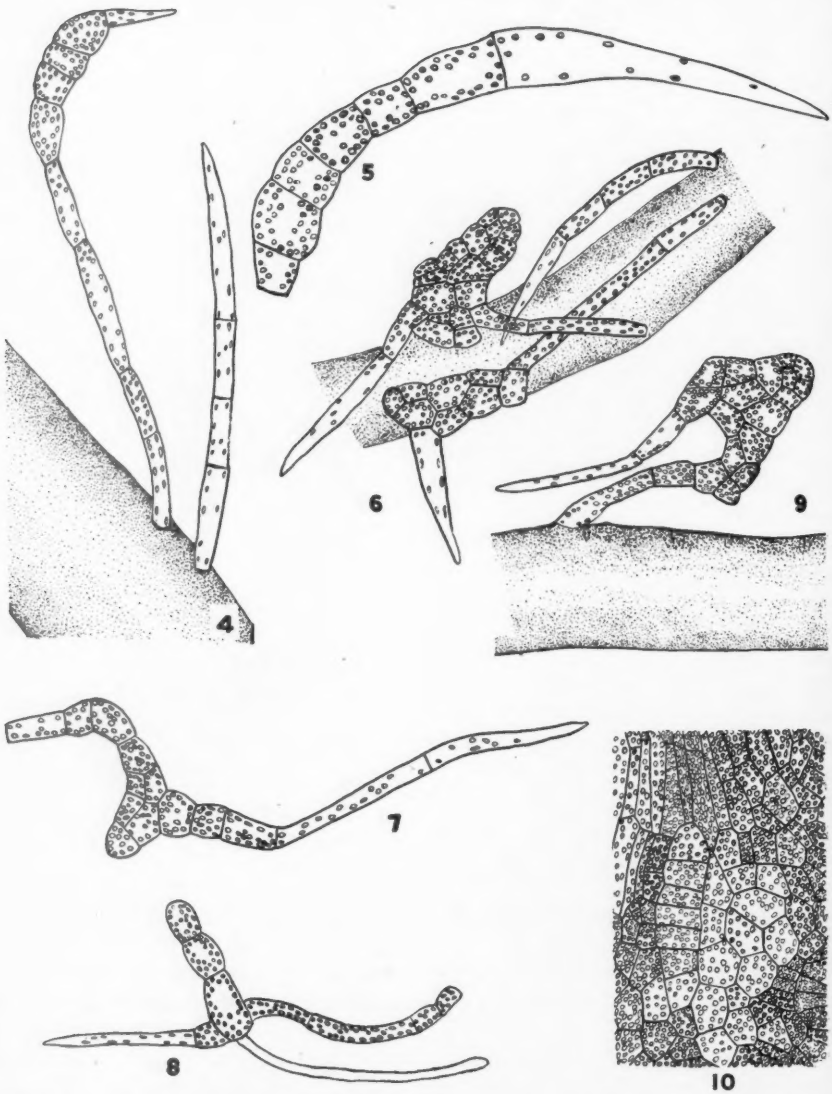


FIGS. 4–10.—*Tectaria* species. Fig. 4, beginning of aposporous gametophyte from one or more cells of one of the two hairs borne by petiole of leaf. Fig. 5, hair produced by petiole; six of the seven cells have become gametophyte. Fig. 6, two young prothallia produced aposporously from hairs of petiole of leaf. Each gametophyte possesses an apical cell. Fig. 7, young aposporously produced gametophyte removed from petiole of leaf of sporophyte. One basal cell and two at opposite end not involved in formation of gametophyte. Fig. 8, young gametophyte produced from only one cell of a hair; rhizoid also formed. Fig. 9, gametophyte produced by cells of a hair. Basal and terminal portion of hair have become nearly parallel in development of gametophyte, which shows well-defined apical cell. Fig. 10, portion of lamina of young leaf of sporophyte. Clearly defined lighter region and some of adjoining portions composed of cells distinctly gametophytic in character.

## LITERATURE CITED


1. ANDERSSON, IRMA, The genetics of variegation in a fern. Jour. Genet. **13**:1–11. 1927.
2. ANDERSON-KOTTÖ, IRMA, Variegation in three species of ferns (*Polystichum angulare, Lastrea strata*, and *Scolopendrium vulgare*). Leits. Ind. Abst. Vererb. **56**:115–201. 1930.
3. ———, The genetics of ferns. Bibl. Genet. **8**:269–294. 1931.
4. FARLOW, W. G., An asexual growth from the prothallium of *Pteris cretica*. Quart. Jour. Micr. Sci. **14**:226–273. 1874.
5. MOORE, G.T., Methods for growing pure cultures of algae. Jour. Appl. Micros. **6**:2309–2314. 1903.
6. STEIL, W. N., Studies in some new cases of apogamy in ferns. Bull. Torr. Bot. Club **45**:93–108. 1918.
7. ———, A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. **33**:109–132. 1919.
8. ———, Vegetative reproduction and aposporous growths from the young sporophyte of *Polypodium iriodes*. Bull. Torr. Bot. Club **48**:203–205. 1921.
9. ———, New cases of apogamy in certain homosporous leptosporangiate ferns. BOT. GAZ. **95**: 164–167. 1933.
10. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. **5**:433–453. 1939.


# PECULIAR ANTHEROZOIDS OF A SPECIES OF CHARA

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In August, 1935, a species of *Chara* was found about 50 miles north of Milwaukee in a pool fed by an artesian well. Some of the plants were brought into the laboratory, where attempts were made to obtain the motile antherozoids. Collections of this species were made by Mr. KARL MILLER and the writer for several years from the pool and from a number of other places near Milwaukee. It was always found growing in cold water, often in a running stream.

All efforts to obtain the motile antherozoids failed until the alga was grown in the University greenhouse in the summer of 1937, when the temperature reached 100° F. for a considerable portion of the day. Under similar temperature conditions, for the past 6 years antherozoids were readily obtained in great numbers.

The technique for obtaining the antherozoids and for preparation of the slides has already been described (9). This species when grown in the laboratory frequently showed many cells of the spermatogenous filaments to be multinucleate. The nuclei were sometimes unequal in size but considerably smaller

FIGS. 1, 2.—Fig. 1, antherozoid of a *Chara* species with double vescicle. Fig. 2, antherozoid with three cilia.

than those in the ordinary spermatogenous cells. In a study of the development of the antherozoid, disturbances in the mitotic process occurred, similar to those described as incomplete mitosis by STEIL (3–8), and more recently as interrupted divisions by a number of investigators, including BLAKESLEE and AVERY (1) and NEBEL and RUTTLE (2). Small cells were also formed in the spermatogenous filaments. In some instances the walls of these smaller cells were oblique to the length of the filament.

Many cells of the filaments aborted, and hence antherozoids were not produced. Undoubtedly disturbances in the nuclear and cell division caused the irregularities in the cells and also their abortion.

Two unusual features were observed occasionally in the mature antherozoids. One of these is the double vesicle. An antherozoid (fig. 1) with a vesicle of this nature was fixed a short time after it had assumed its swimming motions, as is shown by the fact that the vesicle remained intact. No antherozoids with double anterior ends were observed. Another unusual feature is associated with the number of cilia, three or four being present (fig. 2). The ciliary band, partially removed from the anterior end of the body on which it is superimposed, shows the origin of the cilia, two of which are equal in length. The other cilium is of considerably greater length. Such peculiar antherozoids apparently have not heretofore been reported.

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LITERATURE CITED

1. BLAKESLEE, A. F., and AVERY, A. G., Methods of inducing doubling of chromosomes in plants by treatment with colchicine. Jour. Hered. **28**:393–411. 1937.
2. NEBEL, B. R., and RUTTLE, M. L., The cytological and genetical significance of colchicine. Jour. Hered. **29**:3–9. 1938.
3. STEIL, W. N., A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. **33**:109–132. 1919.
4. ———, Nuclear and cell phenomena in the tapetum of *Botrychium virginianum*. Abstract, Bot. Soc. Amer. 1930.
5. ———, Some phenomena in ferns. Abstract, Bot. Soc. Amer. 1931.
6. ———, Incomplete nuclear divisions, not amitosis in the tapetum of some homosporous eusporangiate ferns. Science **77**:606–607. 1933.
7. ———, Incomplete nuclear and cell division in the tapetum of *Botrychium virginianum* and *Ophioglossum vulgatum*. Amer. Jour. Bot. **22**:409–425. 1935.
8. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. **5**:433–453. 1939.
9. ———, Antherozoids of some of the Characeae. BOT. GAZ. **102**:623–628. 1941.

# CYTOGENETIC STUDIES ON TALINUM AND PORTULACA

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## Introduction

Members of the Portulacaceae have received relatively little attention from cytologists. SUGIURA (**7, 8, 9**) found n = 9 in *Portulaca pusilla*, n = 26 in *P. oleracea* var. *sativa*, and n = 18 in *P. marginata*. *P. grandiflora* has been reported to have n = 9 by TJEBBES (**11**), OKURA (**4**), and BLACKBURN (see TISCHLER, **10**). HAGERUP (**2**) found two varieties of *P. oleracea*, one with a haploid number of 9, and *P. oleracea gigas* with n = 27. COOPER (**1**) found n = 27 in *P. oleracea*.

Two species of *Talinum* have been counted, *T. patens* (n = 12) by BLACKBURN (10) and SUGIURA (7), and *T. purpureum* (n = 12) by SUGIURA (7).

Before the outbreak of the present war a cytotaxonomic study of *Portulaca* and *Talinum*, as well as other members of the family, was begun. Since the study is now interrupted, this paper is only a preliminary report and is confined to the preceding more easily grown genera.

MATERIAL AND METHODS.—As cytological material, these genera are unfavorable. Their succulent nature prohibits the use of leaf smears. Root tips are usually scarce. A suitable method for obtaining ample root-tip material consists in removal of the plant from the soil and allowing the roots to dry for a few hours. After drying, the plants are potted in sand, watered well, and placed in any situation where the transpiration rate is relatively high. Abundant root tips are produced in 12–24 hours.

The murky, heavily staining character of the cytoplasm also does not allow adequate chromosomal preparations with some of the usual techniques. Aceto-carmine root-tip smears were impractical, partly because of the tendency for the cytoplasm to stain but primarily because the cells are so shaped as to make it impossible to obtain polar views of the metaphase plate. Furthermore, divisions are infrequent. The use of sectioned material is therefore more practical.

The following procedure was found effective in avoiding preparations with heavily stained cytoplasm. All material was fixed in Belling's modification of Navashin's fluid, and after dehydration and imbedding according to LA COUR'S (3) schedule, was sectioned at 10 $\mu$. Newton's crystal-violet-iodine staining schedule was used, except that a 45-minute hydrolysis in 10% HCl at 60° C. was introduced just prior to staining. After hydrolysis, it was washed in running water for 10–15 minutes and subsequently stained. Destaining was carried out rapidly, less the stain be removed entirely. This method brings out the chromosomes in sharp contrast to a light background. An additional advantage lies in the fact that in some cases, where a chromosome plate is not quite clear, it may be flattened and spread somewhat, as is permitted by WARMKE'S (12) section-smear method. Drawings were made with camera lucida at table level.

Many of the species of *Talinum* and *Portulaca* were obtained through the courtesy of Dr. J. T. BALDWIN of the University of Michigan. Acknowledgments are also due Dr. O. E. WHITE of the Blandy Experimental Farm and Dr. V. L. CORY of the Texas Agricultural Experiment Station for collections. In most cases the original species descriptions were used to verify the species identification made by the collector.

## Results

The chromosome numbers are listed in tables 1 and 2. Figures 1–16 show root-tip chromosomes of these species. All species of *Talinum* which were counted fit into a polyploid series, from a haploid number of 12 to one of 36. In the cortical area of the root tips of *T. parviflorum* are polyploid cells showing 96 chromosomes. Such cells are frequent here but were not found in other species.

The chromosomes of *Portulaca* show much greater variation among the species examined than do those of *Talinum*. The chromosomes of the various species differ markedly in size; they are also generally larger than those of *Talinum*. The species of *Portulaca* examined cannot be classi-

fied into a regular polyploid series as in *Talinum*.

There is no record of any interspecific hybridization in either genera, although *T. parviflorum* and *T. teretifolium* and twenty-five attempts to cross *T. aurantiacum* and *T. parviflorum* were unsuccessful.

TABLE 1

| Species | Source | Diploid no. | Previous count | Investigator |
|---|---|---|---|---|
| Talinum | | | | |
| appalachianum Wolf | Eight-mile Creek, St. Bernard, Ala. | 24 | | |
| parviflorum Nutt. | Springdale, Ark. | 48 | | |
| paniculatum Gaertn. | Pearce Seed Co., Moorestown, N.J. | 24 | 24* | Blackburn (1934), Sugiura (1936) |
| paniculatum Gaertn. | Montevideo Botanical Gardens, Montevideo, Uruguay | 24 | | |
| paniculatum Gaertn. | Coimbra Botanical Gardens, Coimbra, Portugal | 24 | | |
| mengesii Wolf | St. Bernard, Ala. | 24 | | |
| teretifolium Pursh. | Wake Forest, N.C. | 48 | | |
| teretifolium Pursh. | Stone Mountain, Ga. | 48 | | |
| teretifolium Pursh. | Bibb County, Ala. | 24 | | |
| aurantiacum Engelm. | Sonora, Tex. | 48 | | |
| variegata | Palmdale, Fla. | 72 | | |
| triangulare Wild. | Coconut Grove, Fla. | 72 | | |
| species | Hidalgo County, Tex. | 24 | | |

* Previous count of *T. paniculatum* reported in literature as a count of *T. patens*. These two species are, however, considered synonymous.

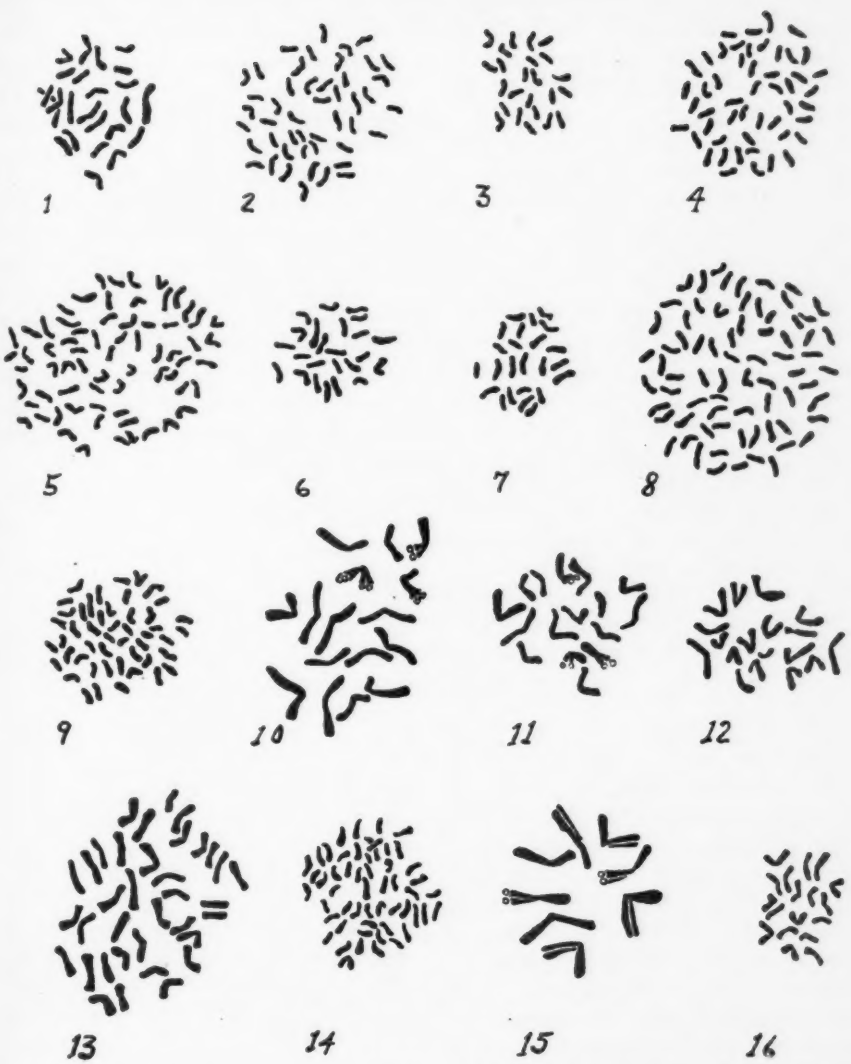
TABLE 2

| Species | Source | Diploid no. | Previous count | Investigator |
|---|---|---|---|---|
| Portulaca | | | | |
| grandiflora Hook. | Wood Seed Co., Richmond, Va. | 18 | 18 | Tjebbes (1928), Okura (1933), Blackburn (1934) |
| oleracea L. | Blandy Experimental Farm, Boyce, Va. | 54 | 54 | Cooper (1935) |
| pusilla | Royal Botanical Gardens, Stockholm, Sweden | 18 | 18 | Sugiura (1936) |
| pilosa L. | Daytona Beach, Fla. | 16 | | |
| smallii Wils. | Wake Forest, N.C. | 16 | | |
| marginata HBK. | Pearce Seed Co., Moorestown, N.J. | 36 | 36 | Sugiura (1936) |
| species | Springdale, Ark. | 8 | | |

*Portulaca grandiflora* has been considerably used for genetical work. Crosses between some of the species were attempted. Fifty crosses between *P. grandiflora* and *P. marginata* were made, twenty-five using the latter as the female parent and twenty-five the reciprocal. No seed was set. Fifty attempts to cross

## Discussion

The genus *Talinum* was first established by ADANSON in 1763. In the monograph of the genus by VON POELLNITZ (5), forty-seven species are recognized. Most of the species are distributed throughout the southwestern United States and Mexico, with a few




Figs. 1–16.—Root-tip chromosomes: Fig. 1, *Talinum* sp.; fig. 2, *T. parviflorum*; fig. 3, *T. appalachianum*; fig. 4, *T. teretifolium*; fig. 5, *T. triangulare*; fig. 6, *T. paniculatum*; fig. 7, *T. mengesii*; fig. 8, *T. variegata*; fig. 9, *T. aurantiacum*; fig. 10, *Portulaca smallii*; fig. 11, *P. pilosa*; fig. 12, *P. grandiflora*; fig. 13, *P. marginata*; fig. 14, *P. oleracea*; fig. 15, *Portulaca* sp.; fig. 16, *P. pusilla*. Figs. 1, 15, ×3200; others, ×2400.

species extending farther north and east. A few species are also found throughout Africa and Australia, but the genus is typically American.

The group may be divided into those forms with terete leaves, those with broad leaves, and an intermediate category with linear leaves. Too few species have been examined to determine conclusively whether there is any tendency toward a correlation of chromosome number and these taxonomic divisions of the genus. In all likelihood there is none, since such species as *T. paniculatum* and *T. triangulare*, two of the broad-leaved forms, are closely related yet exhibit chromosome number differences of a greater degree than the differences between these and terete-leaved species. The chromosome numbers of this genus have apparently little value in determining the eventual taxonomic position of the species; that must be based more or less completely upon morphological criteria.

The species listed in table 1 as *T. variegata* is not recognized by VON POELLNITZ, nor has it been possible to find any taxonomic description elsewhere. Horticulturally this plant is known either as *T. variegata* or as a variety of *T. paniculatum*. The plant differs from *T. paniculatum*, however, not only by its variegation but also by the thicker, more succulent leaves and the less vigorous, more open habit of growth. The chromosome number is also threefold that of *T. paniculatum*. These differences seem to warrant description of the plant as a distinct species.

The genus *Portulaca* has also been monographed by VON POELLNITZ (6), who recognizes 104 species. These are distributed throughout the world, but their greatest concentration occurs in South America. Far too few species have been examined cytologically to warrant definite conclusions, but some general considerations may be indicated. The Portulacas may be divided into three groups, characterized by broad, lanceolate, or terete leaves. The two broad-leaved species which have been counted have a higher chromosome number than those of the other two groups. Also, the numbers indicate that the evolution of the genus has involved chromosomal mutations—such as duplications and deficiencies—concurrent with polyploidy, as contrasted with *Talinum*, the evolution of which probably involved only simple polyploidy.

Two species, a *Talinum* from Hidalgo County, Texas, and a *Portulaca* from Springdale, Arkansas, have not been identified. The *Talinum* is a broad-leaved form related to *T. paniculatum*. The *Portulaca* belongs to the terete-leaved group. A thorough search of the literature has failed to reveal a species description which adequately fits in either case. It would seem, therefore, that both plants are new species. It is hoped that a description can be published in the future.

Attention is particularly called to this *Portulaca* from Springdale, Arkansas. This species might well be adapted as material for class study or in experimental work where a species with a small number of chromosomes morphologically distinguishable is desired.

Appreciation is expressed for guidance and constructive criticism rendered by DR. O. E. WHITE and DR. LADLEY HUSTED of the University of Virginia during the progress of this work.

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LITERATURE CITED

1. Cooper, D. C., Microsporogenesis and the development of the male gametes in *Portulaca oleracea*. Amer. Jour. Bot. **22**: 453–457. 1935.
2. Hagerup, O., Über Polyploidie in Beziehung zu Klima, Ökologie, und Phylogenie. Hereditas **16**: 19–40. 1932.
3. La Cour, L., Improvements in plant cytological technique. Bot. Rev. **3**: 241–258. 1937.
4. Okura, E., A haploid plant in *Portulaca grandiflora* Hook. Jap. Jour. Bot. **7**: 19. 1933.
5. Poellnitz, Karl von, Monographie der Gattung *Talinum* Adans. Repert. Spec. Nov. Fedde. **35**: 1–34. 1934.
6. ———, Versuch zur einer Monographie der Gattung *Portulaca* L. Repert. Spec. Nov. Fedde. **37**: 240–320. 1934.
7. Sugiura, T., A list of chromosome numbers in angiospermous plants. II. Proc. Imp. Acad. Tokyo **12**: 144. 1936.
8. ———, Studies on chromosomes in higher plants with special reference to cytokinesis. I. Cytologia **7**: 544–595. 1936.
9. ———, Studies on chromosomes in higher plants with special reference to cytokinesis. II. Cytologia Fujii Jub. Vol. **2**: 845–849. 1937.
10. Tischler, G., Pflanzliche Chromosomen-Zahlen. Tabulae Biologicae **12**: 57–115. 1937.
11. Tjebbes, K., The chromosome numbers of some flowering plants. Hereditas **10**: 328–332. 1928.
12. Warmke, H. E., A section-smear method for plant cytology. Stain. Technol. **16**: 9–12. 1941.

# EFFECTS OF HIGH SALT CONCENTRATIONS ON GROWTH OF BEAN PLANTS

HUGH G. GAUCH AND CECIL H. WADLEIGH[1]

## Introduction

An earlier experiment (**11**) at this laboratory showed that beans are one of the most sensitive plants to a high concentration of salt. There was little quantitative difference in growth responses to added chloride and to sulphate salts if these were compared on an isosmotic basis. There was a definite tendency, however, toward premature senescence of the older leaves on the plants grown on a substrate containing a high concentration of chloride salts, a condition not shown in cultures with a high concentration of sulphate salts. The leaves of the latter seemed deeper green than those of the controls.

In this earlier experiment, specific responses to various cations were unobservable, since no one cation predominated in the added mixture of calcium, magnesium, and sodium salts. Although under natural conditions the salts in saline soils are almost always mixtures of several cations and anions, it is difficult to ascertain the specific effects of ions from a study of such mixtures. The present study, with single salts added to a basal nutrient solution, was initiated to determine the specific effects of relatively high concentrations of calcium, magnesium, sodium, chloride, and sulphate on plant growth. The bean plant was selected for this investigation.

Methods.—Germinated Red Kidney bean seeds were placed on a paraffined gauze suspended over a tray of basal nutrient solution. Three days later the seedlings were transferred to water-culture equipment (**2**). The containers held 13 liters of solution, which was constantly aerated with carbon-tube aerators. The osmotic concentration was 0.5 atm. (tap-water salts included). The original pH was 5.7, maintained at pH 6.0 by the addition of small amounts of HCl to chloride series and $H_2SO_4$ to sul-

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phate series. The basal nutrient solution in each container had the following composition (me./l.):

| Ca | Mg | Na | K | Cl | $SO_4$ | $H_2PO_4$ | $NO_3$ |
|---|---|---|---|---|---|---|---|
| 5.9 | 2.7 | 2.1 | 4.1 | 1.8 | 4.3 | 1.5 | 7.0 |

0.5 p.p.m. of B, Mn, and Fe, added as boric acid, manganese chloride, and ferric citrate, respectively.

Twenty-four hours after the seedlings were transplanted to the jars, those which were to receive added salts were given the initial salt increment—an amount sufficient to raise the osmotic concentration of the solution one atm. Cultures which were to have more than one increment were given sufficient to increase the osmotic concentration one atm. each day until the desired level was reached. Table 1 lists the salts studied and the number of milliequivalents per liter of each at the various osmotic concentrations. Owing to the very low solubility of $CaSo_4$, it was impossible to study this salt in the range of concentrations employed in this experiment.

TABLE 1

MILLIEQUIVALENTS OF SALT ADDED TO EACH LITER OF BASAL NUTRIENT SOLUTION TO GIVE TOTAL OSMOTIC CONCENTRATIONS OF 1.5, 2.5, 3.5, AND 4.5 ATM.

| SALT | TOTAL ATM. OSMOTIC CONCENTRATION* | | | |
|---|---|---|---|---|
| | 1.5 | 2.5 | 3.5 | 4.5 |
| $Na_2SO_4$ | 39 | 78 | 117 | 156 |
| NaCl | 24 | 48 | 72 | 96 |
| $CaCl_2$ | 32 | 64 | 96 | 128 |
| $MgCl_2$ | 34 | 68 | 102 | 136 |
| $MgSO_4$ | 70 | 144 | 225 | 312 |

* Osmotic concentration of basal nutrient solution, 0.5 atm.

In a series of concentrations of each salt added to basal nutrient solution, the relationship between osmotic concentration and specific electrical conductance was determined, so that frequent periodic determinations of concentration could be made by conductance measurement.

The experiment was started November 27, 1941, and the plants were harvested December 29, when the first flower buds were beginning to open. The plants were divided into (*a*) leaves, (*b*) stems (plus petioles and hypocotyl), and (*c*) roots. Green and dry weights were obtained.

The plants were grown in December, a time of year characterized by comparatively low light intensity. Admittedly, the quantitative and qualitative responses to salt would be different under high light and high temperature conditions. BROYER and HOAGLAND (1) have called attention to the important role of light and photosynthesis in determining the carbohydrate and salt status of the plant, which in turn conditions the salt uptake. In another current experiment attempting to use the bean plant as a "biological yardstick" for integrating all the climatic factors affecting growth, plantings are being made each week and the plants harvested 3 weeks from date of planting. The "standard" plants grown during the course of this experiment (December) weighed less than half as much as those grown in June of the following year.

## Results

The appearance of the plants just prior to harvesting is shown in figure 1. The quality and quantity of growth produced in the presence of the magnesium salts differed radically from that of isosmotic concentrations of the other salts. There were, however, some specific responses to each salt.

$Na_2SO_4$ SERIES.—The quality and quantity of this plant growth was very similar to that in the NaCl and $CaCl_2$ series (fig. 1). The primary leaves were

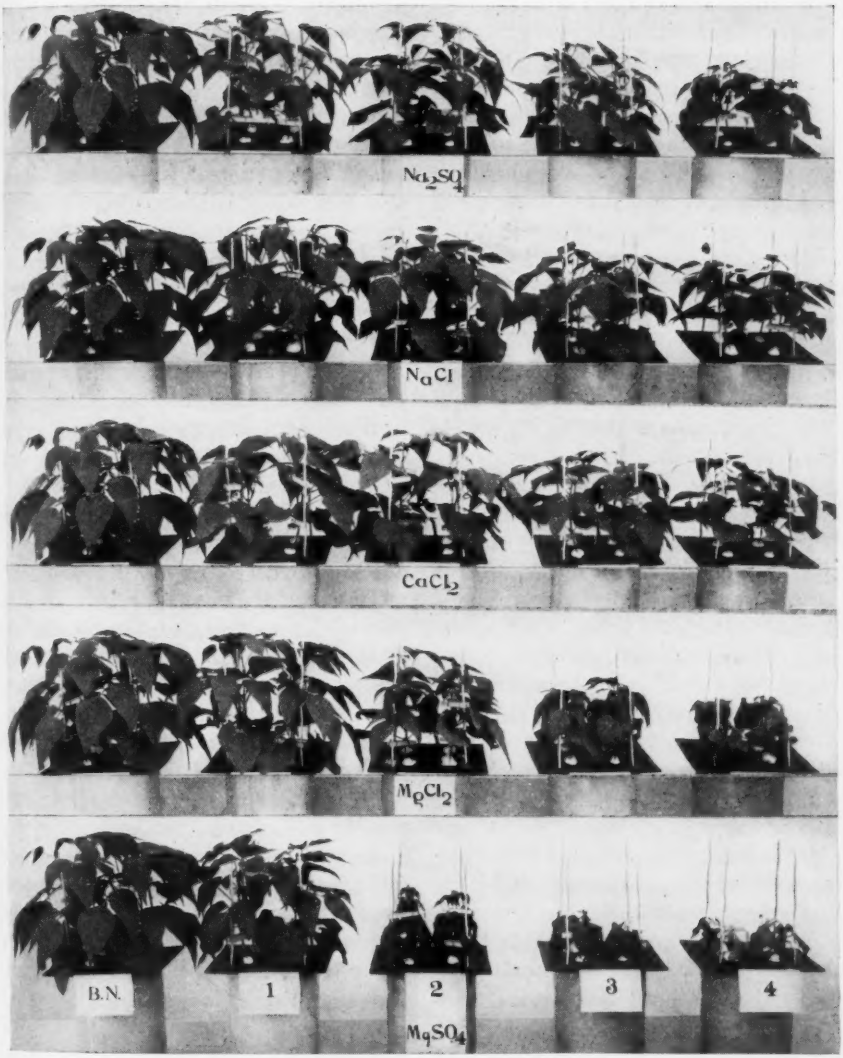


FIG. 1.—Appearance of plants at time of harvest. Figures on jars indicate atm. osmotic concentration of added salts; basal nutrient salts, 0.5 atm.

of the same color as those of the control plants, but the higher the concentration of $Na_2SO_4$ the more pronounced was the marginal and tip burn, resulting in brown necrotic tips and margins. The younger trifoliate leaves were darker green than those of the controls. The addition of the salt had no obvious effect on the type of growth or on the color of the roots.

NaCl SERIES.—At isosmotic concentrations, these plants resembled those of the $Na_2SO_4$ series in general appearance. In contrast with plants receiving $Na_2SO_4$, the primary leaves of these plants were yellow-green—lighter in color than those of the controls. As in the $Na_2SO_4$ series, the younger trifoliate leaves were darker green than those of the control plants. There were no observable specific effects of this salt on the roots.

$CaCl_2$ SERIES.—At isosmotic concentrations, these plants were similar in appearance to the $Na_2SO_4$ and NaCl series. The primary and first trifoliate leaves were yellow-green, while the second trifoliate leaf and the younger trifoliate leaves were yellow-green to green. In general, the plants were lighter in color than those of the controls of the $Na_2SO_4$ and NaCl series.

$MgCl_2$ SERIES.—In the presence of one atm. of added salt, the quantity and quality of growth was comparable with that of the three previous series. With twice this amount of salt, the symptoms were specific. The primary leaves and the first and second trifoliate leaves were very light in color (yellow-green), and the symptoms developed on these primary leaves were characteristic of those usually designated as "chloride burning." This burning differs from that which occurs with high concentrations of sulphate (for example, $Na_2SO_4$), in that it is not restricted to the tips and margins but frequently occurs in isolated patches on the primary leaves. Often there is some tip burn in addition to the isolated necrotic patches. Abortion of tertiary lateral roots was prevalent, and those tertiaries which did not early abort made a growth of only $\frac{1}{4}$–$\frac{1}{2}$ inch. When high magnesium concentration results in abortion of tertiary lateral roots, the loci of their primordia are represented by only brown dots.

At the 2-atm. concentration of $MgCl_2$ there was evidence of magnesium toxicity on the above-ground portion of only one out of twelve plants. In this one plant there was a slight necrosis (browning) in the pulvini directly below the base of the leaflets of the second trifoliate leaf.

At the next highest concentration (3 atm.) the magnesium toxicity symptoms were more pronounced. There was moderate to severe necrosis of all pulvini directly below the base of the blades. The primary leaves were curled under slightly at the tips, and the leaf blades seemed thicker and were leathery to the touch. The interveinal tissue was elevated. The roots were tan in color, and there was practically no development of tertiary roots. Soon after the terminal growing points of the primary and secondary roots became inactive or were killed, the root primordia just above the main root tips for a distance of 10–15 mm. became active and grew 2–10 mm. before the tips of these laterals in turn became inactive or died. This resulted in a rosette of roots near the tip of many of the primary and secondary roots.

At the highest concentration (4 atm.) the symptoms were even more acute. There were three very chlorotic trifoliate leaves on a much-shortened stem axis. The roots were brown, with many rosettes, and there was complete inhibition of tertiary roots.

$MgSO_4$ SERIES.—In the presence of 1

atm. of added salt, plant weights were about 10% less than with the other four salts. Distinct magnesium toxicity symptoms were in evidence, even at this lowest concentration. There was some necrosis in the pulvini at the base of the primary leaf blades and at the base of all trifoliate leaflet blades. At this concentration of $MgSO_4$ the necrosis in the pulvini was of limited extent and affected only a portion of the cortex on the abaxial surface of the pulvini. On about one-fourth of the plants there was browning or necrosis of the smaller veins on the third trifoliate leaf. The color of the roots was light tan, as in the 1-atm. $MgCl_2$ plants, and there was nearly complete inhibition of tertiary roots.

With 2 atm., the dry weight of the plants was 40% that of the 1-atm. plants and about 29% that of the controls. At the 1-atm. level the terminal leaflets of all trifoliate leaves measured more than 2 inches in breadth; but with twice as much salt, none of the terminal leaflets of any of the four trifoliate leaves measured 2 inches. The stem axis was much shortened (fig. 1). Necrosis in the pulvini regions was severe and in many of the primary leaves extended from the pulvinus at the base of the blade downward on to the petiole and centrifugally to the main veins of the primary leaves. There was a slight wilted appearance to the primary leaves. The roots were tan to brown, and there were practically no tertiary roots. There were a few rosettes of roots on some of the primary and secondary roots near the tips.

At the second-highest concentration (3 atm.) the dry weight of the plants was about 23% of that of the 1-atm. and about 16% of that of the control plants. In most cases there were only two trifoliate leaves, and the terminal leaflets of these did not attain 1 inch in breadth. All previously described symptoms were much more acute, including the "wilting" of the primary leaves.

With 4 atm., only one plant out of twelve was alive at the time the experiment was concluded.

Although the preceding descriptions have dealt with the appearance of the plants on the day preceding harvesting, the magnesium toxicity symptoms on the tops—and especially on the roots—were in evidence within a day after all solutions had been brought to full concentration. Naturally these symptoms became more pronounced with time.

### Growth data

Average dry weights and percentage dry matter are given in table 2. At isosmotic concentrations, very similar amounts of top and root growth occurred in the presence of NaCl, $CaCl_2$, and $Na_2SO_4$ (fig. 2). In view of the different cations and anions involved in these three salts, the amount of growth was obviously not related to cation or anion specificity; rather the data show a linear relationship between growth and total osmotic concentration of the solution. Other work (**3, 5, 6, 7, 8, 9, 11**) has also indicated that for most salts or mixtures of salts the amount of growth is closely related to the osmotic concentration of the solution in contact with the roots.

The various salts diversely affected the percentage dry matter in the tops and roots (fig. 3). There was also a segregation of effects, that is, in the tops there was a grouping of the curves of the chloride salts *vs.* the sulphate salts. Thus in the tops the degree of "succulence" was apparently determined by the type of anion (Cl *vs.* $SO_4$). In the roots there tended to be a definite grouping, but in

TABLE 2

INFLUENCE OF SINGLE SALTS (ADDED TO BASAL NUTRIENT SOLUTION) ON DRY WEIGHT AND PERCENTAGE DRY MATTER OF BEAN PLANTS

| Salt added | Total osmotic concentration (atm.) | Dry weight per plant (gm.) | | | | Dry matter (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | Leaves | Stems | Roots | Whole plant | Leaves | Stems | Roots |
| None | 0.5* | 3.71 | 1.57 | 1.21 | 6.49 | 10.94 | 8.85 | 5.45 |
| $Na_2SO_4$ | 1.5 | 3.20 | 1.38 | 1.09 | 5.67 | 11.46 | 9.71 | 5.29 |
| | 2.5 | 2.50 | 1.08 | 0.92 | 4.50 | 12.00 | 10.25 | 5.30 |
| | 3.5 | 1.77 | 0.75 | 0.69 | 3.21 | 12.51 | 10.86 | 5.68 |
| | 4.5 | 1.21 | 0.55 | 0.54 | 2.30 | 13.29 | 11.64 | 6.20 |
| NaCl | 1.5 | 3.24 | 1.39 | 1.13 | 5.76 | 10.62 | 9.34 | 5.32 |
| | 2.5 | 2.62 | 1.20 | 0.97 | 4.79 | 10.88 | 9.87 | 5.24 |
| | 3.5 | 2.11 | 0.91 | 0.74 | 3.76 | 10.12 | 10.44 | 5.20 |
| | 4.5 | 1.52 | 0.68 | 0.58 | 2.78 | 10.96 | 11.05 | 5.32 |
| $CaCl_2$ | 1.5 | 3.13 | 1.35 | 1.00 | 5.48 | 10.96 | 9.93 | 6.16 |
| | 2.5 | 2.70 | 1.16 | 0.93 | 4.79 | 10.36 | 10.43 | 6.66 |
| | 3.5 | 1.85 | 0.81 | 0.60 | 3.26 | 10.95 | 11.04 | 6.62 |
| | 4.5 | 1.58 | 0.66 | 0.54 | 2.78 | 11.29 | 11.62 | 6.71 |
| $MgCl_2$ | 1.5 | 3.13 | 1.40 | 0.98 | 5.51 | 10.14 | 9.07 | 6.27 |
| | 2.5 | 2.17 | 0.86 | 0.55 | 3.58 | 10.85 | 10.13 | 7.04 |
| | 3.5 | 1.19 | 0.47 | 0.31 | 1.97 | 10.66 | 10.89 | 8.08 |
| | 4.5 | 0.79 | 0.23 | 0.18 | 1.20 | (14.09) | 12.39 | 7.61 |
| $MgSO_4$ | 1.5 | 2.79 | 1.15 | 0.75 | 4.69 | 12.21 | 9.99 | 7.67 |
| | 2.5 | 1.13 | 0.43 | 0.32 | 1.88 | 16.38 | 13.79 | 9.50 |
| | 3.5 | 0.67 | 0.23 | 0.16 | 1.06 | 17.67 | 15.53 | 8.73 |
| | 4.5 | 92% mortality; no plants harvested | | | | | | |

* Basal nutrient.

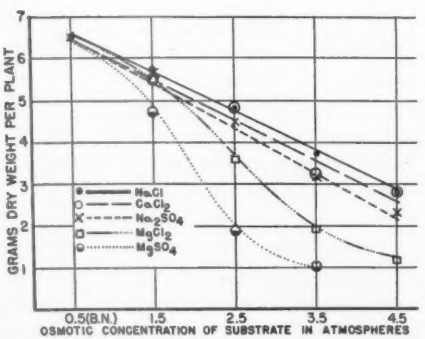


FIG. 2.—Average dry weight of bean plants as influenced by salts added to basal nutrient solution.

this case the comparison is monovalent sodium *vs.* the divalent cations, calcium and magnesium. In a previous paper (5) it was shown that when bean plants were subjected to high concentrations of NaCl, sodium predominated over calcium (on a milliequivalent basis) only in the roots—the only part of the plant in which there was a marked accumulation of sodium. The low percentage dry matter in the roots (high "succulence") associated with sodium and the high percentage dry matter associated with calcium or magnesium agree with the well-known effects of sodium *vs.* calcium and magnesium on the degree of hydration of colloidal material. As for the effect of chloride *vs.* sulphate on the percentage dry matter in the tops, HAYWARD and LONG (9) reported increased succulence in tomatoes associated with chloride and decreased succulence (higher percentage dry mat-

ter) associated with sulphate salts. The results of this experiment are in agreement with their findings.

## Discussion

In the light of advances in technique within the past decade or two, most of the early work on the effect of various singly applied salts on the growth of plants was characterized by certain inadequacies. Usually only very small volumes of solution were available to the plant, and often the solution was unchanged and unaerated. The work of BROYER and HOAGLAND (1) and others has recently shown the necessity of adequate aeration for the process of salt absorption by plants. In many cases the individual salts have been added to distilled water only, and in the absence of all the macroelements and microelements it is difficult to apply or evaluate the data thus obtained. Those who added the salt to distilled water only were of necessity forced to conduct short-term experiments, and there are few data on plants carried to the flowering stage or to maturity. In some cases the investigator replaced one or more of the major salts of the basal nutrient with an added salt, thus using a solution deficient in one or more of the macroelements. Inasmuch as the amount of salt added varied, the degree of substitution of added salt for regular constituents of the basal nutrient solution was simultaneously a variable.

In the present study, the individual salts were added to a basal nutrient solution known to be adequate for at least a near-optimal growth of the bean plant. There were 13 liters of solution per container (supporting four plants), and the solution was continuously well aerated. Determinations were made of the osmotic concentration of the solutions, and the concentrations were maintained as nearly constant as possible.

Inasmuch as the necessary nutrient elements were supplied to all cultures in this experiment, any variations in the growth responses were believed to be strictly attributable to the individually added salts. Since certain specific-ion

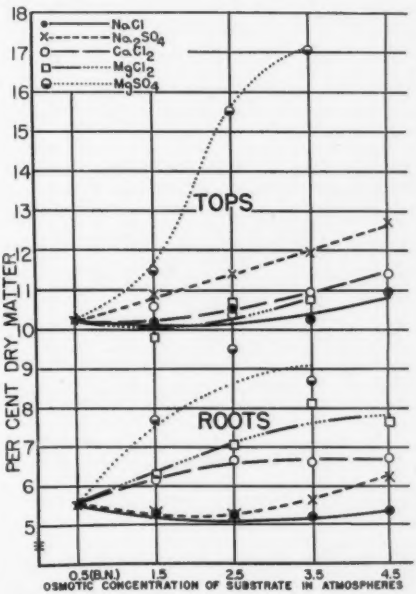


FIG. 3.—The influence of single salts (added to basal nutrient solution) on percentage dry matter in tops and roots of bean plants.

plant symptoms were obtained with the individual salts, these specific symptoms might be of diagnostic value in appraising the quality and something of the quantity of salt in the soils. It is not an uncommon situation to find in an irrigated soil a predominant accumulation of one salt, such as sodium chloride or sodium sulphate. In a study of seventeen representative western soils, MAGISTAD and REITEMEIER (10) found several soils having solutions in which the magnesium concentration exceeded that of

calcium. Although very low in total salt content, one of the more important agricultural soils in California contains more magnesium than calcium. The usual case, of course, is that when saline accumulations occur there is a mixture of accumulated salts. In a previous paper (13) it was pointed out that sodium chloride has an effect on nitrogen metabolism which is distinguishable from that produced by calcium chloride. It was possible to correlate the observed effects on nitrogen metabolism with (*a*) the uptake and concentration of sodium *vs.* calcium in the various parts of the bean plant and (*b*) the known effects of these two cations on the hydration of colloids. The factors determining salt uptake are not yet fully elucidated, however, and certainly the process is a complex one, as has been pointed out by BROYER and HOAGLAND (1).

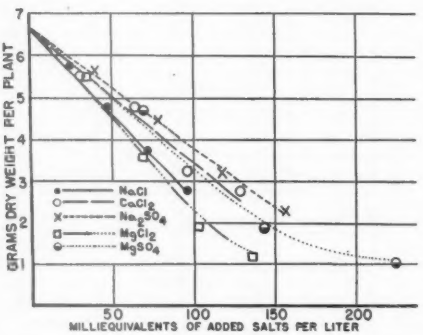


FIG. 4.—Average dry weight of bean plants as influenced by salts added to basal nutrient solution.

The marked difference in the effect of Mg upon the growth response as compared with that of Na or Ca is the striking feature of this study. It is evident in table 2 and in figure 3 that growth in the presence of a magnesium salt is markedly reduced as compared with growth in the presence of $NaCl_2$, $CaCl_2$, or $Na_2SO_4$. Inasmuch as this disparity appears with either $MgCl_2$ or $MgSO_4$, it is clearly a matter of specific cationic (magnesium) toxicity. Magnesium toxicity has been previously reported for wheat (12) and for Red Kidney bean (4).

In the present study, at isosmotic concentrations there was greater growth reduction in the presence of $MgSO_4$ than with $MgCl_2$. In terms of milliequivalents per liter, there was slightly more than twice as high a concentration of magnesium in the $MgSO_4$ solutions as in the $MgCl_2$ solutions. With respect to the specific action of magnesium, it is pertinent to consider the disparity in the amount of growth made in the presence of $CaCl_2$ *vs.* $MgCl_2$ (fig. 2) where essentially equal concentrations in equivalents of ions were involved (table 1). Thus it is evident that there is cationic specificity in the case of magnesium which operates in addition to the osmotic concentration effect of the solution. There is a rather close relationship between the concentration of magnesium (me./l.) and growth depression, but it must be assumed that other factors (for example, osmotic concentration, effect of associated anion on rate of uptake of magnesium, etc.) are also operative.

When growth of the plants in the magnesium salts is plotted against milliequivalents of salt per liter (fig. 4), $MgCl_2$ results in greater reduction in growth than does $MgSO_4$. Inorganic analyses of all samples have not been completed as yet, but a few were made, and these show that at iso-equivalent concentrations of $MgCl_2$ and $MgSO_4$, appreciably more magnesium was taken up when in association with chloride than when with sulphate. Higher concentrations of chloride were found in the leaves of $MgCl_2$ plants than in either the $CaCl_2$ or NaCl plants, which finding

would seem to be correlated with the pronounced "chloride burning" symptoms reported earlier in this paper for $MgCl_2$ plants. Over and above the effect of osmotic concentration on growth, high concentrations of magnesium thus produce specific symptoms on the tops and roots of bean plants.

It should be stressed that the specific symptoms noted in this paper are for the bean; other plants might not exhibit similar symptoms. The use of the bean plant as an indicator in studies of saline soils is suggested.

## Summary

1. Red Kidney bean plants were grown to the flowering stage in aerated solution culture with basal nutrient solution and in this solution with various amounts of individual salts added.

2. The added salts were $Na_2SO_4$, NaCl, $CaCl_2$, $MgCl_2$, and $MgSO_4$. The effect of these separate salts on plant growth was studied in concentrations of 1.5, 2.5, 3.5, and 4.5 atm. osmotic concentration, including the 0.5 atm. concentration of basal nutrient.

3. At isosmotic concentrations very similar amounts of plant growth occurred in the NaCl, $CaCl_2$, and $Na_2SO_4$ series, but there was marked depression of growth with $MgCl_2$ and $MgSO_4$.

4. For most salts and mixtures of salts there tends to be a linear relationship between growth and osmotic concentration, expressed in atmospheres. The greatly depressed growth of plants in the presence of $MgCl_2$ or $MgSO_4$, as compared with that in NaCl, $CaCl_2$, and $Na_2SO_4$, is attributed to the specific toxicity of magnesium. Magnesium toxicity symptoms, as they occurred on the bean plant, are described.

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## LITERATURE CITED

1. Broyer, T. C., and Hoagland, D. R., Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Amer. Jour. Bot. **30**:261–273. 1943.

2. Eaton, F. M., Plant culture equipment. Plant Physiol. **16**:385–392. 1941.

3. ———, Water uptake and root growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. **16**:545–564. 1941.

4. Gauch, H. G., Response of the bean plant to calcium deficiency. Plant Physiol. **15**:1–21. 1940.

5. Gauch, H. G., and Wadleigh, C. H., The influence of saline substrates upon the absorption of nutrients by bean plants. Proc. Amer. Soc. Hort. Sci. **41**:365–369. 1942.

6. Gauch, H. G., and Magistad, O. C., Growth of strawberry clover varieties and of alfalfa and Ladino clover as affected by salt. Amer. Soc. Agron. **35**:871–880. 1943.

7. Hayward, H. E., and Long, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. Bot. Gaz. **102**:437–462. 1941.

8. ———, and ———, Vegetative responses of the Elberta peach on Lovell and Shalil rootstocks to high chloride and sulfate solutions. Proc. Amer. Soc. Hort. Sci. **41**:149–155. 1942.

9. ———, and ———, Some effects of sodium salts on the growth of the tomato. Plant Physiol. **18**:556–569. 1943.

10. Magistad, O. C., and Reitemeier, R. F., Soil solution concentrations at the wilting point and correlation with plant growth. Soil Sci. **55**:351–360. 1943.

11. Magistad, O. C., Ayers, A. D., Wadleigh, C. H., and Gauch, H. G., The effect of salt concentration, kind of salt, and climate on plant growth in sand cultures. Plant Physiol. **18**:151–166. 1943.

12. Trelease, S. F., and Trelease, Helen M., Magnesium injury of wheat. Bull. Torr. Bot. Club **58**:127–148. 1931.

13. Wadleigh, C. H., and Gauch, H. G., Assimilation in bean plants of nitrogen from saline solutions. Proc. Amer. Soc. Hort. Sci. **41**:360–364. 1941.

# MYCORHIZAS OF FILBERT AND WALNUT TREES IN OREGON ORCHARDS[1]

C. E. SCHUSTER,[2] R. E. STEPHENSON,[3] AND WILLIAM EVENDEN[4]

## Introduction

Walnut and filbert trees in orchards of Oregon show the development of mycorhizas in sufficient abundance to justify a survey to determine the types involved and their prevalence. In 1934, studies of roots of filbert and walnut trees in orchards of the Willamette Valley in Oregon were begun by SCHUSTER and STEPHENSON. The investigation included determination of the distribution of the roots within each soil horizon. In some soils roots were restricted to very limited depths; and apparently typical root absorptive regions (root-hair zones) were inadequate for satisfactory plant development, as the quantity of roots recovered from the soil was very small compared with that found for some other fruit trees.

FRANK (2) proposed as early as 1885, and also subsequently, that both endotrophic and ectotrophic forms of mycorhizas were capable of modifying root absorptive regions to the advantage of the host plants. Later, HATCH (4) and MITCHELL, FINN, and ROSENDAHL (7) showed that ectotrophic mycorhizas are capable of aiding in the nutrition of specific forest trees in definite soil types. The influence of an endophytic fungus on the germination of orchid seed has been shown (5) to be related to the nutrition of the developing embryo. Other cases of assumed benefit or detriment from ectotrophic or endotrophic mycorhizas have largely remained unproved.

METHODS.—In determining the prevalence of mycorhizas, several hundred root samples were taken at random from nine filbert and twelve walnut orchards, representative of most of the soil types on which these trees are grown in the Willamette Valley. Since, according to SCHUSTER and STEPHENSON (10), the greatest concentration of roots occurs generally in the first or second foot, samples were collected for the most part from the upper 2 feet of soil, with sufficient material from lower depths to establish the presence or absence of mycorhizas.

The roots were washed free of loose soil in a large-mesh sieve and stored in water for several hours to loosen adhering soil particles. They were then inclosed by fine-mesh wire screen and washed thoroughly with a strong stream of tap water. The desired parts were removed from the washed material with forceps and scissors and kept submerged in water or in killing and fixing solution at all times.

In preparation for histological study, the material was fixed in weak chrom-acetic fixative for 48 hours (1) or for 24 hours (9), then dehydrated in dioxan and imbedded in paraffin in the usual way and cut 8–10 $\mu$ thick. Preparation for staining was according to GALIGHER (3) and the staining was in Delafield's haematoxylin.

## Results

### ECTOTROPHIC MYCORHIZAS OF CORYLUS AVELLANA L.

Ectotrophic mycorhizas are so generally present on filbert roots (fig. 1) that

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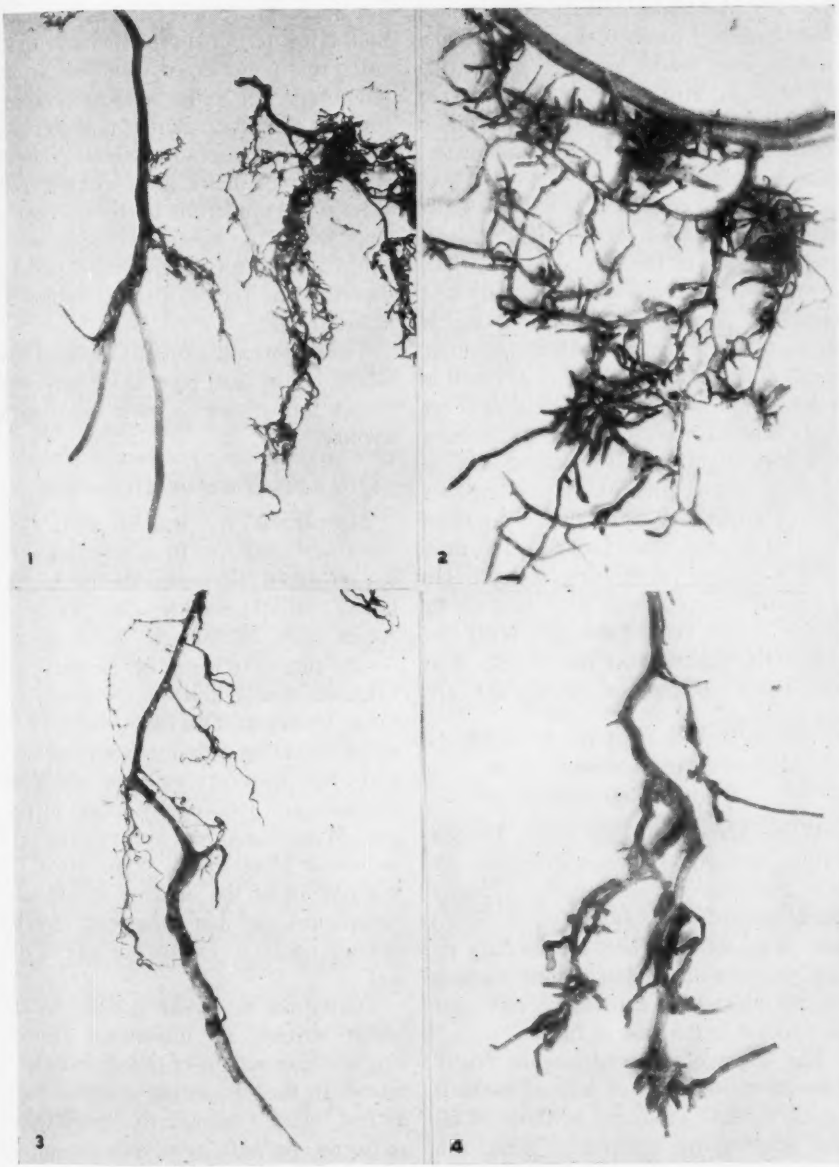


FIGS. 1–4.—Fig. 1, mycorhizal and nonmycorhizal roots of filbert; root at left has two long nonmycorhizal tips; remainder are ectotrophic mycorhizas. Fig. 2, ectotrophic mycorhizal roots of filbert; note excessive branching. Fig. 3, mycorhizal and nonmycorhizal roots of walnut. Long terminal root is nonmycorhizal; others are old endotrophic mycorhizas. Fig. 4, endotrophic mycorhizal roots of walnut; note clubbing and distortion.

their effects on root development cannot be determined under orchard conditions. Studies were made, however, of the differences in structure between mycohizal and noninfected roots. Ectotrophic mycorhizas have not been found in the case of walnut.

Mycorhizal condition of roots is indicated by cessation of terminal growth and clubbing of the laterals (fig. 2). In turn, these laterals make only limited terminal growth, a condition which seems to encourage formation of another series of short laterals. The final result is a dense, much-branched system of short roots, whereas noninfected roots are long and only slightly branched. The surface of each root is covered by a mantle of fungus growth. Within this is another layer of hyphae, the Hartig net, formed by the hyphae penetrating intercellular spaces of the epidermal layer to a depth of one or two cells. From this layer develops the mantle just mentioned. The root tissues below the Hartig net are noninfected.

## Endotrophic mycorhizas of Juglans regia L.

While these data are from Persian walnut orchards, the roots examined are from seedlings of the northern California black walnut (*Juglans hindsii* Sarg.) used as rootstock. There are no data regarding roots of Persian walnut seedling trees. Endotrophic mycorhizas have not been found in the case of filbert.

The effect of the endotrophic mycorhiza on the growth of walnut roots is similar to that produced in those of filbert affected by ectotrophic fungi, in that terminal growths are shortened and laterals clubbed and shortened (figs. 3, 4). A larger proportion of noninfected roots is found than in the case of filbert, but the fungi are never absent from more than a few terminal growths, and apparently this noninfected condition is only temporary. In young endotrophic mycorhizas of walnut there is no outer layer of fungus but instead epidermal cells devoid of root hairs and without cork. Within the epidermis is the infected region, where the hyphae are intracellular —in contrast to the condition found in filbert, where the ectotrophic hyphae are intercellular.

The endotrophic fungus or fungi have sterile nonseptate mycelia of phycomycetous form, with at least two sizes of hyphae.

## Occurrence of mycorhizas

Mycorhizas on walnut and filbert roots were collected from orchards growing on Aiken, Olympic, Melbourne, Salkum, and Willamette clay loam; on Aiken, Sites, Willamette, Newberg, and Amity silty clay loam; on Newberg and Chehalis loam; and on Newberg fine sandy loam soil. This list includes all the series of better soils for nut production and some mediocre soils on which nut orchards are occasionally found in Oregon. Mycorhizas were always present on walnut or filbert roots, regardless of the soil type or of the amount of available phosphorus, soluble potassium, soluble calcium, organic matter, or pH of the soil.

During the first year of study by the senior writers, no unaffected growing tips of either walnut or filbert roots were found. In the succeeding year, however, a few white, apparently noninfected roots on walnuts were present at all times of the year after early summer. In that investigation no histological studies were made.

No attempt was made to identify the

fungi; the study was concerned only with their presence and distribution. Presence of the fungus or fungi may be explained in two ways. Filbert trees imported from Europe have been the foundation stock for most orchards in the Pacific Northwest. FRANK (2), LECOMTE (6), and PEYRONEL (8) record mycorhizas on *Corylus avellana* L. of Europe, and these may have been introduced on such imported stock. Also, since young trees are generally propagated by layerage from bearing trees or from stools especially used for the purpose, it is probable that further dissemination occurred. However, mycorhizas have been found on the native hazel, *Corylus californica* (Rose), indicating that the same or related species of fungi are indigenous. In the case of the walnut, the fungus or fungi are probably indigenous, since practically all the rootstock material consisted of seedlings produced in the Willamette Valley, grown from nuts locally produced or from California.

## Discussion

On shallow infertile soils, trees are small, low in production, and have a greater rate of mortality than trees on the deep fertile soils. In these shallow soils the roots are confined to the upper levels, and roots of walnut or filbert trees growing thereon are almost completely infected with mycorhizas. In deep fertile soils the total quantity of roots per tree is much greater than in shallow soils, but in the upper levels of both types of soil the quantity of roots is approximately equal. In deep soils the mycorhizas are more widely and deeply distributed than in shallow soils.

Regardless of soil type, degree of fertility, or physical or chemical condition of the soil, mycorhizas are equally abundant in all soils on which walnut and filbert trees are growing. Under field conditions there is no evidence that in the deep fertile soils mycorhizas are either necessary or detrimental to the development of large productive trees, or that mycorhizal development on weak devitalized trees growing on shallow infertile soil has hastened or otherwise promoted their decline. Since some investigators claim the function of root hairs for both ectotrophic and endotrophic mycorhizal hyphae, it is assumed that this role is played in the case of walnut and filbert, regardless of type, depth, or physical or chemical quality of the soil.

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## LITERATURE CITED

1. CHAMBERLAIN, C. J., Methods in plant histology. 4th ed. Univ. of Chicago Press. 1924.
2. FRANK A. B., Ueber die physiologische Bedeutung der Mycorhiza. Ber. deutsch. Bot. Ges. 6:248–268. 1888.
3. GALIGHER, A. E., Essential of practical microtechnique. Laboratory of microtechnique. Albert E. Galigher, Berkeley, California. 1934.
4. HATCH, A. B., The physical basis of mycotrophy in *Pinus*. Black Rock Forest Bull. 6. 1937.
5. KNUDSON, L., Non-symbiotic germination of orchid seeds. BOT. GAZ. 73:1–25. 1922.
6. LACOMTE, H., Note sur le mycorhize. Bull. Soc. Bot. France 34:38–39. 1887.
7. MITCHELL, H. L., FINN, R. F., and ROSENDAHL, R. O., The relation between mycorhizas and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock Forest Papers 1:10. 1937.

8. PEYRONEL, B., Nouveaux cas de rapports mycorhiziques entre phanérogames et Basidiomycètes. Bull. Soc. Mycol. France 37:143–146. 1921.

9. SCHUSTER, C. E., Methods for softening filbert buds imbedded in paraffin. BOT. GAZ. 102:815–818. 1941.

10. SCHUSTER, C. E., and STEPHENSON, R. E., Soil moisture, root distribution, and aeration as factors in nut production in western Oregon. Ore. Agr. Exp. Sta. Bull. 372. 1940.

## BORON DEFICIENCY AND THE ASCORBIC-ACID CONTENT OF TOMATOES

C. B. LYON AND R. Q. PARKS

A series of investigations concerned primarily with the effects of mineral nutrition on the ascorbic-acid content of tomatoes have been completed at this laboratory. Large variations in the supply of calcium, potassium, magnesium, nitrate, sulphate, and phosphate produced relatively little effect on the ascorbic-acid content of tomato fruits (3). Similarly, lack of copper, iron, manganese, zinc, and molybdenum in the substrate resulted in no change of any practical importance in the vitamin content of fruits (4). In the latter study, specialized Pyrex apparatus was used which precluded a study of boron deficiency. It is the purpose of this note to record the ascorbic-acid content of tomatoes from boron-deficient plants grown in sand culture. The effects of a toxic supply of the micronutrients on the vitamin content of tomatoes will be reported elsewhere.

On May 1, 1943, seed of an inbred strain of Bonny Best tomatoes was germinated in the greenhouse in small crocks filled with pure quartz sand. The seedlings were supplied with nutrient solution of the following composition (p.p.m.): Ca, 285; K, 223; Mg, 69; N, 168; S, 144; P, 70; Fe, 5.0; Mn, 0.5; Mo 0.5; Zn, 0.05; Cu, 0.02. Eighteen seedlings were transplanted into 2-gallon glazed crocks with one seedling per crock on May 20, and all plants were placed outdoors. Nine plants were supplied with this nutrient solution throughout their life span; the remaining nine plants were supplied with the same nutrient containing in addition 0.5 p.p.m. of B. The first four fruits to mature on each plant were harvested on the morning of the day that complete color change had occurred and immediately analyzed for ascorbic acid by methods previously described (3). All plants were harvested August 21, when they were 112 days old.

The comparison of various characteristics for boron-deficient and control plants is given in table 1. Significantly less growth and fruitfulness occurred when boron was omitted from the nutrient medium. In addition, severe deficiency symptoms similar to those obtained in previous investigations were observed on vegetative parts of the plant (1, 2) and on fruits (2, 5). When all plants were harvested at the conclusion of the experiment, leaflet material of each plant was analyzed in duplicate for boron content. Leaflets from deficient plants averaged 7.6 $\pm$ 1.19 p.p.m. of boron on a dry-weight basis, while those from control plants contained 91.8 $\pm$ 3.30 p.p.m.

The average of twenty-six analyses of boron-deficient fruits was 25.9 $\pm$ 0.86 mg. of ascorbic acid per 100 gm. of fresh weight, while the average of thirty-six

analyses of the control fruits was 26.6 ± 0.58 mg. Within the limits of error there is no statistically significant difference in these means.

The lack of boron in this experiment resulted in less growth and fruitfulness, accompanied by severe deficiency symptoms with less boron in vegetative parts of the plant, but did not significantly affect ascorbic-acid content of fruits.

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TABLE 1

Measurements of growth and fruitfulness giving treatment means together with their standard errors

| Plant character | Treatment* | |
|---|---|---|
| | Boron deficiency | Control |
| I. Vegetative growth | | |
| Height of vine (cm.) | 67.7± 5.74 | 109.6± 4.39 |
| Fresh weight of vine (gm.) | 200.5± 23.10 | 356.0±18.63 |
| Dry weight of vine (gm.) | 37.1± 4.60 | 68.7± 3.18 |
| Percentage dry matter | 18.2± 0.75 | 19.4± 0.50 |
| Dry weight of roots (gm.) | 4.7± 0.49 | 9.0± 0.70 |
| II. Fruitfulness | | |
| Total fresh weight ripe fruit per plant (gm.) | 331.4±106.71 | 831.7±63.76 |
| Number of fruit ripened per vine | 4.9± 1.21 | 9.7± 0.81 |
| Size ripe fruit (gm./fruit) | 65.9± 14.77 | 88.5± 6.91 |
| Number of immature fruit per vine at end of experiment | 0.2± 0.22 | 9.3± 1.38 |
| Total fresh weight of immature fruit per vine (gm.) | 4.3± 4.30 | 499.1±70.48 |
| III. Analytical results | | |
| Boron content of leaflets (γ/gm. dry wt.) | 7.6± 1.19 | 91.8± 3.30 |
| Ascorbic-acid content of fruit (mg./100 gm. fresh wt.) | 25.9± 0.86 (26) | 26.6± 0.58 (36) |

* In any inter-treatment comparison (except for ascorbic acid), 16 degrees of freedom are available. When $t = 2.12$, $P = 0.05$; when $t = 2.92$, $P = 0.01$. In ascorbic-acid comparison, number of analyses for each mean is indicated in parentheses.

## LITERATURE CITED

1. Johnston, E. S., and Dore, W. H., The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. **4**:31–62. 1929.
2. Johnston, E. S., and Fisher, P. L., The essential nature of boron to the growth and fruiting of the tomato. Plant Physiol. **5**:385–392. 1930.
3. Hamner, K. C., Lyon, C. B., and Hamner, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. Bot. Gaz. **103**:586–615. 1942.
4. Lyon, C. B., Beeson, K. C., and Ellis, G. H., Effects of micronutrient deficiencies on growth and vitamin content of the tomato. Bot. Gaz. **104**:495–514. 1943.
5. Raleigh, G. J., Fruit abnormalities of tomatoes grown in various culture solutions. Proc. Amer. Soc. Hort. Sci. **37**:895–900. 1939.

# ANATOMICAL RESPONSES OF TOMATO STEMS TO VARIATIONS IN THE MACRONUTRIENT ANION SUPPLY

C. B. LYON AND C. R. GARCIA

## Introduction

Numerous studies have been made on the anatomy of the tomato plant (4). Many of these studies, however, have been concerned primarily with descriptive or developmental anatomy, and the anatomy of the plant as it may be correlated with physiological responses has received comparatively little attention. KRAUS and KRAYBILL (6) correlated differences in the anatomy of tomato stems with nitrogen supply and the carbohydrate metabolism of the plant. HAYWARD and LONG (5) found differences in the cross-sectional area of tissue systems in tomato stems to be related to toxic concentrations of NaCl and $Na_2SO_4$ in the nutrient medium. LYON (7) reported hereditary variations in the extent of the tissue systems in tomato stems. The relative areas of these systems in three strains were not altered by additions of $Na_2SO_4$ in toxic quantities to the nutrient medium.

The present study was undertaken to examine anatomical differences in tomato stems which result from variations in the relative proportions of macronutrient ions supplied to the plant and to correlate observed differences with the vegetative and fruitful condition of the plant. The results with respect to variations in the nitrate, sulphate, and phosphate supplies are reported in this paper.

## Material and methods

The material used was obtained from the 1941 experiment of HAMNER, LYON, and HAMNER, described in detail elsewhere (3). Briefly, an inbred strain of Bonny Best variety of tomatoes was planted in the greenhouse May 12, 1941, and the seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand. On July 1, all plants were placed outdoors. The plants were harvested September 10.

The design of the experiment was that of a randomized block (1) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT'S randomization tables (10). Thus, the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the $t$ test (9) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 were accepted as statistically significant that the observed deviations were not due to the errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

In the forty-four solutions considered in this investigation, the relative proportions of nitrate, sulphate, and phosphate were varied, while the total equivalent concentration of these ions was constant. Each solution contained 12.0 milliequivalents per liter of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium. For convenience, the forty-four solutions are represented in an anion triangle (fig. 1), and treatment numbers are assigned to those solutions used. All solutions contained equal amounts of the microelements in the following concentrations:

| | |
|---|---|
| B (as $HBO_3$) | 0.5 p.p.m. |
| Mn (as $MnCl_2 \cdot 4H_2O$) | 0.5 |
| Zn (as $ZnSO_4 \cdot 7H_2O$) | 0.05 |
| Cu (as $CuSO_4 \cdot 5H_2O$) | 0.02 |
| Fe (as ferric citrate) | 5.0 |

When the plants were 120 days old and had received their respective nutrient treatments for 94 days they were harvested, and a section of the stem from the middle internode of each plant was preserved for an anatomical study. The stem sections were fixed with Navashin's solution, and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl-alcohol series and infiltrated with a paraffin-beeswax rubber mixture. Complete cross sections were cut at 10–20 $\mu$ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

Each stem section was projected so that the resulting image was enlarged approximately thirty diameters. The images were centered on a screen marked off with four diameters dividing the stem section into eight equal sectors (fig. 2). The radial measurements of each tissue system on each of the eight radii were recorded. Thus, the mean of eight measurements was used for xylem, phloem, and cortex; the mean of four measurements was used for pith and stem diameter.

In these measurements the internal phloem was disregarded, and the term "pith" includes all cells internal to primary xylem; "xylem" includes all cells from primary xylem to cambium; "phloem" includes all cells from cambium to pericycle; "cortex" includes all cells from endodermis to and including cuticle.

The mean radial measurement of any tissue system for a given stem section was used to calculate the actual area of that tissue system in the cross section.

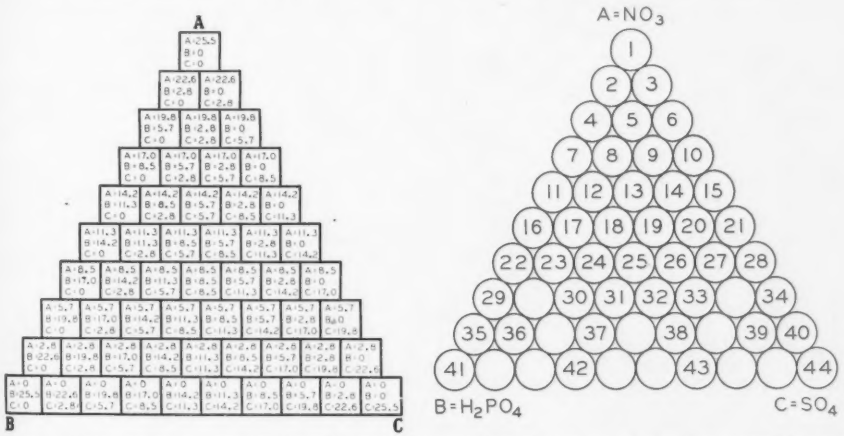


FIG. 1.—Left, anion composition in milliequivalents per liter of nutrient solution for 55 possible treatments. Right, treatments used, with treatment numbers assigned.

Standard formulas developed for circles were employed. It was considered that the variability within a stem attributable to the deviations of the tissue systems from circles was nullified by the use of mean values. Thus, all inter-treatment comparisons consider only differences between stems with respect to areas of the tissue systems. Two types of inter-treatment comparisons were made for each tissue system: (*a*) actual areas were computed and treatment means compared, and (*b*) the percentage cross-sectional area of the stem section occupied by the area of any given tissue system was computed and treatment means compared.

Thus, the actual size as well as the relative amount of tissue in a stem section was evaluated.

## Results

Photomicrographs of cross sections from typical tomato stems in each treatment are given in figure 3. Great differences—which include stem size, tissue system development, starch accumulation, and to some extent cellular anatomy—are demonstrable. These characteristics will be discussed individually.

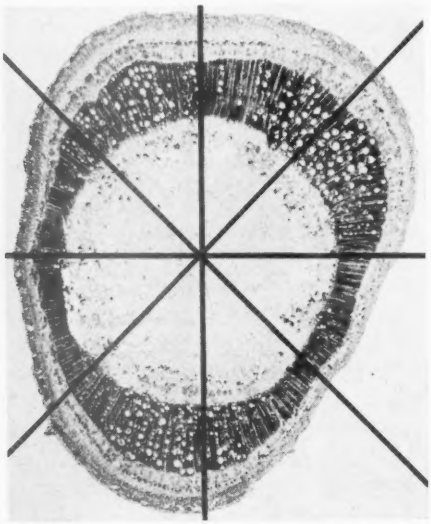

FIG. 2.—Radii used to obtain radial measurements of each tissue system in each stem section. Radii separated by 45° angles.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 1. Several plants were discarded as a result of mechanical injury resulting in a non-orthogonal experimental design. The sources of variation are:

| VARIATION DUE TO | NO. OF DEGREES OF FREEDOM |
|---|---|
| Treatments | 43 |
| Between blocks | 3 |
| Within blocks | 2 |
| Error | 427 |
| Total | 475 |

The substitution of missing values by YATES method (11) did not significantly change the results of analyses presented in table 1, and they are considered valid.

TABLE 1

F VALUES FROM ANALYSES OF VARIANCE FOR ANATOMICAL CHARACTERS OF TOMATO STEM CROSS SECTIONS

| ANATOMICAL CHARACTERS | SOURCE OF VARIATION | | |
|---|---|---|---|
| | Between treatments | Between blocks | Within blocks |
| Percentage | | | |
| Cortex | 4.24* | 1.36 | 0.69 |
| Phloem | 17.68* | 2.75† | 9.78* |
| Xylem | 18.72* | 1.28 | 7.97* |
| Pith | 24.37* | 1.95 | 12.72* |
| Area | | | |
| Cortex | 28.39* | 4.93* | 4.16† |
| Phloem | 39.12* | 6.93* | 8.69* |
| Xylem | 44.43* | 4.65* | 4.49† |
| Pith | 14.43* | 2.17 | 0.75 |
| Stem diameter | 47.33* | 3.92* | 7.13* |

* Highly significant, satisfying statistical requirements for P = 0.01.

† Possibly significant, satisfying statistical requirements for P = 0.05.

It is evident that treatments produced statistically significant differences in all the characters listed. It is also evident that differences in environment—when measured by replications both between and within blocks—produced significant differences in all characters except the percentage cross-sectional area occupied by the cortex and the actual area of the pith. Since replication differences were measurable in the analysis of variance,

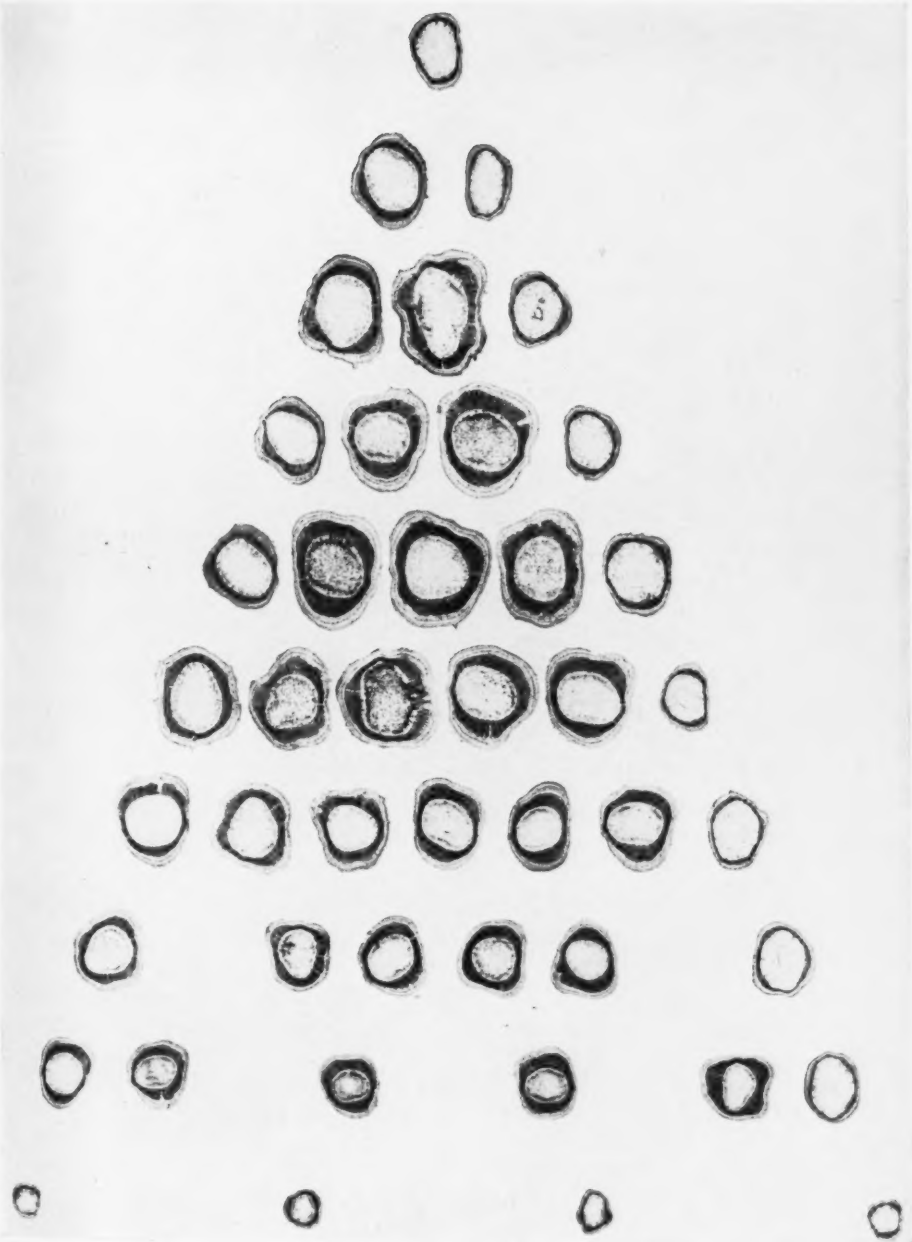

FIG. 3.—Typical cross sections of tomato stems from plants in anion treatments. Treatments correspond to those shown in fig. 1.

and since these differences were not included in inter-treatment comparisons, valid differences exist in all the listed characters which can be ascribed directly to the treatments.

An inter-treatment comparison of stem diameters is given in figure 4. Plants in treatments receiving no nitrate subsequent to the seedling stage had the smallest stems of any in the experiment.

FIG. 4.—Inter-treatment comparison of stem diameters. Treatment no. 9, with actual stem diameter of 10.7 mm., designated 100 and all other treatments related to it. Treatment means, together with standard errors, given.

Significantly larger stems were noted with each successive increment of nitrate supply, and the largest were obtained in treatment no. 9, receiving 17.0 m.e./l. of nitrate in the nutrient solution. However, plants receiving nitrate as the only macronutrient anion (treatment no. 1) compared unfavorably with optimum treatments in the central portion of the triangle with respect to this character. Stem size was significantly less than optimum in all phosphate-deficient treatments and also in all sulphate-deficient treatments.

From figure 5 it is evident that the area of each of the component tissue systems varies in a manner similar to that observed for stem diameter. Without exception, nitrate-deficient plants have the smallest tissue systems of any, and the areas of each of the component tissue systems increase with increments of nitrate supply. Maximum values are obtained in those treatments receiving 17.0 m.e./l. of nitrate in the nutrient medium. Values for phosphate-deficient treatments are significantly less than those for optimum treatments shown in the central portion of the triangle. Little variation in phosphate-deficient treatments as a result of altered nitrate and sulphate supply is indicated. While values for all sulphate-deficient treatments are significantly less than maximum, significant variations occur as a result of differences in nitrate and phosphate supply.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN INDICATED VARIABLES IN CROSS SECTIONS OF TOMATO STEMS

| ANATOMICAL CHARACTERS | AREA | | | STEM DIAMETER |
|---|---|---|---|---|
| | Phloem | Xylem | Pith | |
| Area* | | | | |
| Cortex | +0.977† | +0.950† | +0.493† | +0.966† |
| Phloem | ..... | +0.978† | +0.853† | +0.952† |
| Xylem | ..... | ..... | +0.542† | +0.952† |
| Pith | ..... | ..... | ..... | +0.934† |

* Actual area of indicated tissue system in stem cross section.

† Highly significant, satisfying statistical requirements for P = 0.01.

The correlations involving areas of tissue systems and stem diameter as variables are given in table 2. It is evident that highly significant and positive correlations exist between each of the component tissue systems and stem diameter. The magnitude of the correlation coefficients is unusually high. Thus,

an increase in stem diameter and hence cross-sectional area of stems as a result of treatment is the result of a corresponding increase in the area of each of

While the actual amount of cortex was highly variable as a result of treatment, the percentage cross-sectional area of the stem section occupied by the area of the

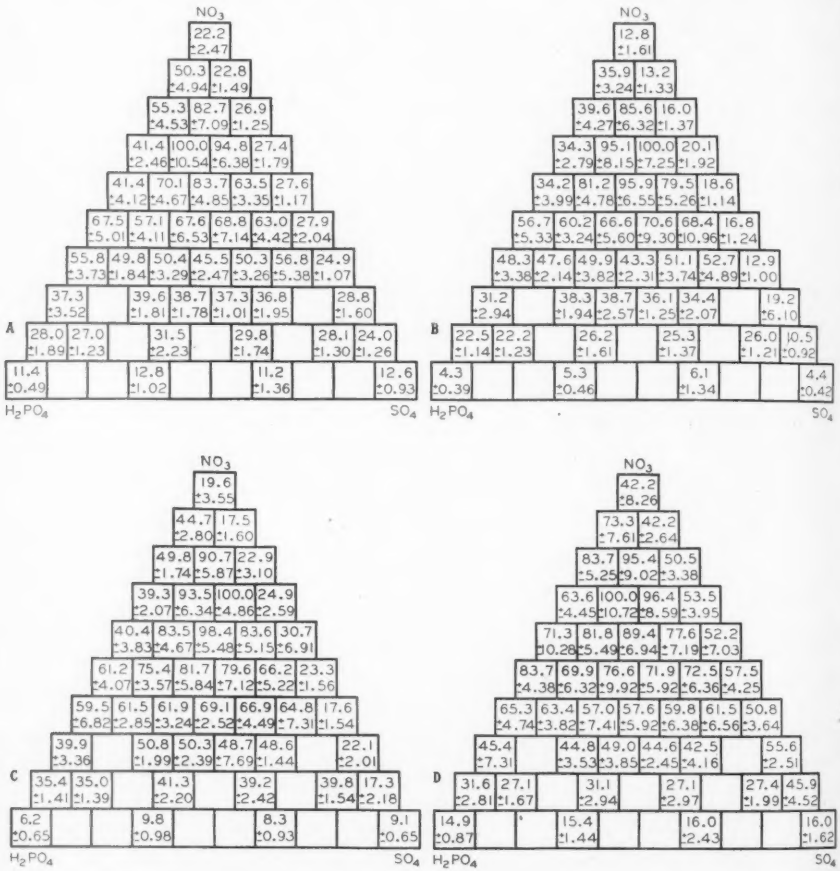


FIG. 5.—Inter-treatment comparison of actual areas of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith, in stem cross sections. Treatment with maximum value designated 100 and all other treatments related to it. Treatment means, together with standard errors, given.

the component tissue systems. The areas of the tissue systems are also inter-correlated among themselves by highly significant coefficients. It is noticeable that two of the correlations involving pith as a variable are less striking in their magnitude.

cortex remained relatively constant (fig. 6). Although the mean differences are statistically significant, as shown by the analysis of variance (table 1), the magnitude of the differences is small and very little correlation with nutrient supply is evident. There is some indication that a

greater percentage of cortex exists in stem sections from nitrate-deficient treatments, but variability is great, as indicated by the excessively high standard errors involved.

with increments of nitrate. Maximum values are obtained in those treatments supplied with 14.2 m.e./l. Both sulphate-deficient and phosphate-deficient treatments are significantly less than maxi-

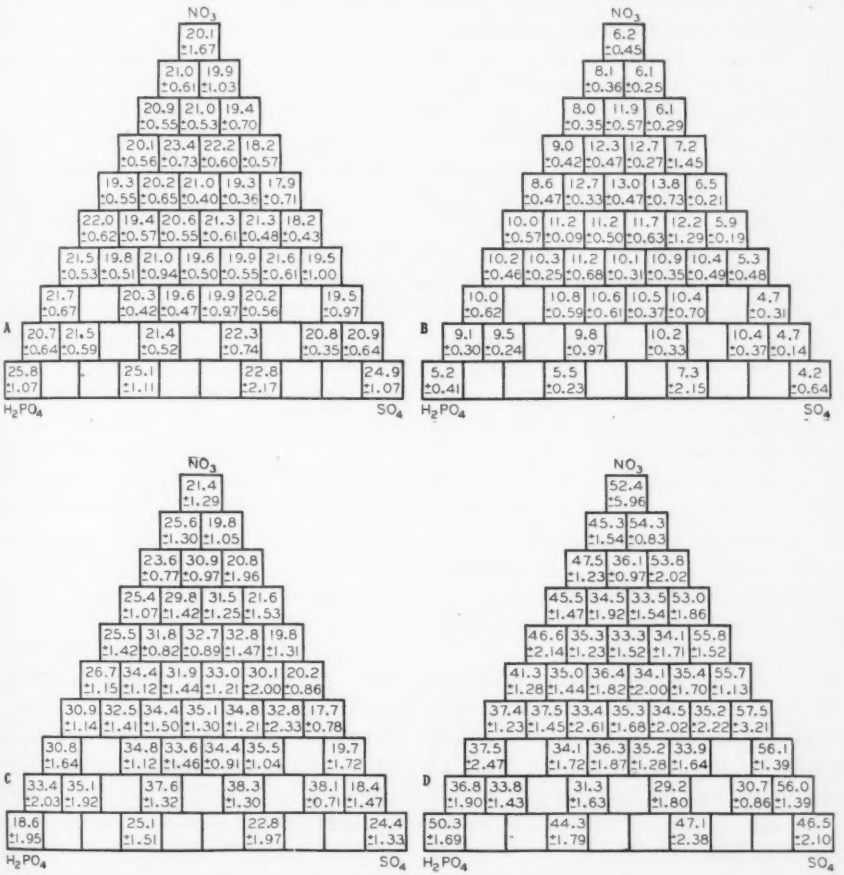


FIG. 6.—Percentage cross-sectional area of stem section occupied by area of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith. Treatment means, together with standard errors, given.

The percentage cross-sectional area in stem sections occupied by the area of the phloem (fig. 6) shows significant differences which can be correlated with treatment. The relative amount of phloem in stem sections is smallest in nitrate-deficient treatments and increases with respect to this characteristic. Thus, not only the actual amount of phloem but the relative amount in each stem section can be correlated with nutrient supply.

The relative amount of xylem in stem sections is smallest in phosphate-defi-

cient but is also small in nitrate-deficient treatments. It is greatest in those treatments receiving only one increment of nitrate in the nutrient medium (2.8 m.e./l.). As greater amounts are supplied to the plant, the relative amount of xylem in the stem section tends to become successively less, although it does not become comparable with values observed for nitrate-deficient stem sections. In sulphate-deficient treatments, a relatively high phosphate and low nitrate supply results in a large relative amount of xylem tissue.

The trends shown in the data for percentage cross-sectional area occupied by pith appear the reverse of those for the relative amount of xylem in stem sections. The greatest relative amount of pith occurs in phosphate-deficient treatments, although the magnitude of the values in nitrate-deficient treatments is also high. The smallest percentage pith was found in stem sections from treatments supplied with 2.8 m.e./l. of nitrate in the nutrient medium. In sulphate-deficient treatments a high nitrate and low phosphate supply results in relatively large amounts of pith.

In these data certain trends seem obvious. Stems from plants supplied with nutrient solutions devoid of nitrate have small amounts of vascular tissue and large amounts of pith. This is also true of phosphate-deficient and to a lesser extent of sulphate-deficient treatments. In treatments supplied with only small amounts of nitrate (2.8 m.e./l.), maximum relative amounts of xylem and minimum relative amounts of pith are present in stem sections. Although the relative amount of phloem tends to increase, the relative amount of xylem in stem sections decreases with increments of nitrate supply. That a unit increase in the relative amount of xylem in a stem section is accompanied by a unit decrease in the relative amount of pith is shown by the highly significant and negative correlation coefficient of -0.971 (table 3). A similar general relationship exists between percentage phloem and percentage pith; and, as would be expected, a significant positive correlation exists between the two components of the vascular system. Since the relative amount of cortical tissue remains constant, no significant correlation with other tissue components of the stem was observed.

One of the most noticeable anatomical differences in the stem sections was the disintegration of central pith cells in all phosphorus-deficient treatments. The remaining pith cells were large, succulent, thin-walled parenchyma with abnormally large intercellular spaces. They were completely devoid of starch. Internal phloem development was at a minimum, with usually three groups of four small sieve tubes and companion cells centrad to the primary xylem points. Both internal and external pericyclic fibers were thin-walled and few in number. Secondary xylem consisted of small thin-walled xylem vessels and wood fibers with undifferentiated parenchymatous cells immediately adjacent to the cambium. Secondary phloem was predominantly parenchyma, and only a few small sieve tubes with companion cells were evident. Cortical parenchyma was similar in appearance to pith parenchyma, with abnormally large intercellular spaces. The development of collenchymatous cells was restricted, and relatively little wall thickening had occurred, especially in those treatments also supplied with small amounts of nitrate. Chlorenchyma was restricted to two or three cell layers immediately internal to the epidermis, and most of these cells had disintegrated.

Central pith cells in nitrogen-deficient

treatments had also disintegrated but less so than in phosphorus-deficient treatments. The parenchymatous pith cells were small in nitrate-deficient treatments and increased in size with increases in nitrate supply. Small accumulations of starch were evident in nitrate-deficient stems immediately centrad to internal phloem, but it did not extend to central pith cells. This resulted in an interrupted ring of starch-filled cells in the outer portion of the pith. The amount of starch accumulated in pith and cortical cells increased as nitrate supply increased, reaching maximum proportions in the central portion of the triangle. Internal pericyclic fibers were practically absent from nitrate-deficient stem sections, but, when present, were extremely small and thick-walled. The number of external pericyclic fibers was not unusual, but they were similar in character to internal fibers. In those treatments receiving 2.8 m.e./l. of nitrate in the nutrient supply, internal and external pericyclic fibers were small but relatively thin-walled, and both size and thickness of wall structure increased with increasing supply. Maximum expression of these characters was noted in treatment no. 5, where 19.8 m.e./l. of nitrate was supplied. Internal phloem formation was negligible in nitrate-deficient stems but increased with increments of nitrate supply, reaching a maximum with respect to number and size of cells in treatment 13 supplied with 14.2 m.e./l. Xylem vessels were small and few in number in nitrate-deficient treatments as contrasted with treatments in the central portion of the triangle. This was the only cellular characteristic of xylem tissue which could be correlated with nitrate supply. Secondary phloem consisted of densely packed small parenchymatous cells in nitrate-deficient treatments, and progressive increases in size and number of cells, as well as differentiation into sieve tubes, companion cells, and phloem fibers were noted with increased nitrate supply. The cortex of nitrate-deficient stems consisted of small parenchyma cells, small relatively thin-walled collenchyma, and a single cell layer of disintegrated chlorenchyma with few chloroplasts. In the central portion of the triangle, the cortex consisted of large parenchyma cells, well-developed and thickened collenchyma, and five or six cell layers of chlorenchyma densely packed with chloroplasts.

TABLE 3

CORRELATION COEFFICIENTS BETWEEN INDICATED VARIABLES IN CROSS SECTIONS OF TOMATO STEMS

| Anatomical characters | Percentage | | |
|---|---|---|---|
| | Phloem | Xylem | Pith |
| Percentage* | | | |
| Cortex | −0.080 | +0.034 | −0.196 |
| Phloem | ..... | +0.812† | −0.870† |
| Xylem | ..... | ..... | −0.971† |

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.

† Highly significant, satisfying statistical requirements for P = 0.01.

No distinguishing characteristics of sulphate-deficient stems were observed, their cellular characteristics being influenced to a greater extent by the relative nitrogen and phosphorus supply than by the lack of sulphate as such.

Stem sections obtained from treatment no. 1, supplied with nutrient solutions containing relatively large amounts of nitrate and no phosphate or sulphate, were of interest. Such characteristics as disintegration of central pith cells, large parenchymatous pith cells with abnormally large intercellular spaces, absence of starch accumulation, relatively few

extremely thin-walled internal and external pericyclic fibers, undifferentiated xylem parenchyma adjacent to the cambium, and disintegration of chlorenchyma were found in these stem sections. These characteristics were also present in phosphate-deficient stems. On the other hand, the stem sections were also characterized by thick-walled xylem vessels and wood fibers, relatively nutritive growth and—to some extent—fruitfulness, it seemed worth while to examine the correlations between actual amounts as well as relative proportions of tissue systems in stem sections with characteristics used as criteria of growth and fruitfulness. The data concerned with growth and fruitfulness of these plants have been previously reported from this laboratory (3, 8). The results

TABLE 4

CORRELATION COEFFICIENTS BETWEEN ANATOMICAL CHARACTERISTICS OF TOMATO STEMS AND CHARACTERS USED AS CRITERIA OF GROWTH AND FRUITFULNESS

| Anatomical characters | Growth | | | | Fruitfulness | | |
|---|---|---|---|---|---|---|---|
| | Ht. of vine | Fresh wt. of vine | Dry wt. of vine | Dry wt. of roots | Total no. fruit/vine | Fresh wt. fruit/vine | Ave. fresh wt. mature fruit |
| Percentage* | | | | | | | |
| Cortex | −0.129 | −0.114 | −0.025 | +0.001 | −0.085 | −0.053 | −0.167 |
| Phloem | +0.912‡ | +0.898‡ | +0.910‡ | +0.896‡ | +0.925‡ | +0.913‡ | +0.925‡ |
| Xylem | +0.802‡ | +0.599‡ | +0.615‡ | +0.652‡ | +0.735‡ | +0.681‡ | +0.765‡ |
| Pith | −0.827‡ | −0.697‡ | −0.712‡ | −0.739‡ | −0.791‡ | −0.755‡ | −0.797‡ |
| Area† | | | | | | | |
| Cortex | +0.771‡ | +0.932‡ | +0.935‡ | +0.893‡ | +0.830‡ | +0.862‡ | +0.809‡ |
| Phloem | +0.517‡ | +0.967‡ | +0.921‡ | +0.935‡ | +0.875‡ | +0.911‡ | +0.840‡ |
| Xylem | +0.871‡ | +0.977‡ | +0.978‡ | +0.961‡ | +0.930‡ | +0.950‡ | +0.902‡ |
| Pith | +0.609‡ | +0.782‡ | +0.784‡ | +0.741‡ | +0.668‡ | +0.693‡ | +0.661‡ |
| Stem diameter | +0.827‡ | +0.921‡ | +0.927‡ | +0.901‡ | +0.866‡ | +0.875‡ | +0.864‡ |

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.

† Actual area of indicated tissue system in stem cross section.

‡ Highly significant, satisfying statistical requirements for P = 0.01.

merous sieve tubes and companion cells in the secondary phloem, and abundant thick-walled collenchyma cells in the cortex. All these characteristics were also present in high-nitrate stem sections and were not typical of phosphate-deficiency conditions.

It has been observed that significant and large differences occurred between treatments, not only with respect to stem diameter and the actual area of the component tissue systems, but also with respect to the relative proportions of the tissue systems in the stem. Since stem size is directly correlated with vegetative growth and fruitfulness of the correlation analysis are given in table 4.

These data show stem diameter as well as actual areas of each of the component tissue systems to be significantly and positively correlated with each of the characteristics used as criteria of growth and fruitfulness. This means that each unit increase in the diameter of stems or in the cross-sectional area of tissue systems was accompanied by a unit increase in growth and fruitfulness of the plants. However, the correlation coefficients also indicate that a unit increase in the percentage of cross-section-

al area in stem sections occupied by vascular tissue is accompanied by a unit increase in growth and fruitfulness of the plants. Those correlations involving percentage phloem are equal in magnitude to those involving area phloem, although those involving percentage xylem are significantly less by FISHER's $Z$ test (2) than those involving area xylem. It is also notable that percentage phloem is correlated to a significantly higher degree with growth and fruitfulness than is percentage xylem. The relative proportion of pith is significantly and negatively correlated with growth and fruitfulness, since a strong negative correlation exists between percentage pith and percentage vascular tissue. The relative proportion of cortex is not significantly correlated with any of the characters of growth and fruitfulness, since the percentage cortex remained relatively constant. Thus, in this experiment greater growth and fruitfulness of the plants were accompanied by increased stem diameter, increase in the cross-sectional area of each of the tissue systems, and increase in the relative proportion of vascular tissue in the stem.

## Discussion

It seems worth while to consider the foregoing results with respect to nitrate supply in terms of the differences reported by KRAUS and KRAYBILL (6). These investigators characterized stems from feebly vegetative plants grown with a limited nitrogen supply as tough and woody. In a study of their internal structure, they pointed out that pith, cortex, and medullary rays were packed with starch. The walls of outer and inner pericyclic fibers were greatly thickened, while collenchyma development was limited to two or three cell layers. In contrast, they characterized stems from vigorously vegetative plants grown with an abundant nitrogen supply as succulent and brittle. Their diameter was large, as a partial result of the greater number and size of pith cells. No starch had accumulated in pith or cortex, and the relative proportion of xylem in the stem sections was greatly reduced. Both chlorenchyma and collenchyma were well developed, with a large number of chloroplasts in cortical and pith cells.

From this description, it is evident that plants grown with a relatively great nitrate supply in this experiment could be classified as feebly vegetative, owing to the large quantities of starch in pith cells, thick-walled outer and inner pericyclic fibers, and the tough woody nature of the stems. It seems apparent that the high light intensities prevalent during this investigation resulted in extensive carbohydrate synthesis, and that nitrate supply was a limiting factor—even in those treatments supplied with relatively large amounts of nitrate. This supposition is further supported by the fact that fruitfulness increased with increments of nitrate supply and was maximum in those treatments supplied with 17.0–19.8 m.e./l.

A lack of phosphate or sulphate in the nutrient medium was associated with less growth and fruitfulness of tomato plants in this experiment (3). However, a relatively small supply of either ion (2.8 m.e./l.) was sufficient to result in maximum growth and fruitfulness, and no response to successively higher increments in the supply of these ions was obtained. It is apparent from the data presented here that differences in cellular characteristics and tissue system development of tomato stems could be closely correlated with growth and fruitfulness of the plants. While anatomical responses could be correlated with a deficient supply of phosphate or sulphate, the re-

sponses could not be correlated with successive increments of phosphate or sulphate when the supply of either ion was in excess of 2.8 m.e./l.

It is of interest also to compare stem sections from plants grown at Riverside, California, in 1940 (7) with those of the present experiment. A large volume of nutrient containing 8.1 m.e./l. of nitrate was provided for each plant, and the plants were automatically irrigated with nutrient solution at hourly intervals during the daytime, with one additional irrigation at midnight. Light intensities were comparable with those of the present experiment. The sections were characteristic of vigorously vegetative stems described by KRAUS and KRAYBILL.

In this triangle, maximum growth and maximum fruitfulness were obtained in the same treatment nos. 8, 9, 13, 14. In the cation triangle, however, which involved variations in the supply of calcium, potassium, and magnesium, a somewhat different situation existed. Using criteria similar to those employed in this investigation, maximum vegetative growth was obtained in treatments supplied with relatively small amounts of calcium, while maximum fruitfulness was obtained in treatments supplied with relatively large amounts of calcium (3). Results of anatomical studies in the cation triangle will be presented at a later date and should help in evaluating the correlations observed here. Further discussion of these results will be delayed until the additional data are available.


## Summary

1. An inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of forty-four nutrient solutions varying in the relative proportions of macronutrient anions were studied in relation to the anatomy of plant stems. Measurements of stem diameter and the actual area of each of the component tissue systems were recorded. The data were reduced and analyzed by statistical methods.

2. Great differences in stem diameter and the actual area of each of the component tissue systems could be correlated with differences in nutrient supply.

3. Differences in the relative proportion of phloem, xylem, and pith in stem sections were correlated with differences in nutrient supply. No differences were found in the relative amount of cortex.

4. Cellular differences in pith parenchyma, xylem vessels and fibers, internal and external phloem, internal and external pericyclic fibers, and cortical cells are described and correlated with nutrient supply.

5. Differences in the anatomy of the tomato stems are significantly correlated with characteristics used as criteria of vegetative growth and fruitfulness.



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## LITERATURE CITED


1. FISHER, R. A., The design of experiments. Oliver & Boyd, London. 1942.
2. ———, Statistical methods for research workers. Oliver & Boyd, London. 1936.
3. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Factors influencing the ascorbic-acid content of the tomato, with special reference to mineral nutrition. BOT. GAZ. 103:586–616. 1942.
4. HAYWARD, H. E., The structure of economic plants. Macmillan, New York. 1938.
5. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. BOT. GAZ. 102:437–462. 1941.
6. KRAUS, E. J., and KRAYBILL, H. R., Vegetation

and reproduction with special reference to the tomato. Ore. Agr. Coll. Exp. Sta. Bull. 149. 1918.

7. LYON, C. B., Responses of two species of tomatoes and the $F_1$ generation to sodium sulphate in the nutrient medium. BOT. GAZ. 103:107–122. 1941.

8. LYON, C. B., BEESON, K. C., and BARRENTINE, M., Macro-element nutrition of the tomato plant as correlated with fruitfulness and occurrence of blossom-end rot. BOT. GAZ. 103:651–667. 1942.

9. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.

10. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge Univ. Press, London. 1927.

11. YATES, F., The analysis of replicated experiments where the field results are incomplete. Empire Jour. Exp. Agr. 1:129. 1933.

## A METHOD OF NUMERICALLY EVALUATING AREAS OF PLANT TISSUE

A. GERALDINE WHITING[1] AND JOHN W. MITCHELL[2]

In order to measure the anatomical characteristics of numbers of plants, a rapid histological schedule resulting in slides showing the tissue areas clearly, and simple methods for measuring regular and irregular areas have been devised. Numerical results obtained by these methods are suitable for statistical analysis.

METHOD.—Samples were collected from specific portions of a plant, such as at given levels of the primary root, the main stem, or at points along a representative branch. These were either sectioned fresh or fixed in a formalin-acetic-alcohol solution and stored for later use. Before sectioning, the preserved material was washed over-night. Sections 40–60$\mu$ thick were cut on the freezing microtome. The sections were placed successively in water, aqueous stain, water, then 25% glycerin, and finally mounted in glycerin jelly. The sections were then placed in a 1% aqueous solution of crystal violet for several minutes, according to the intensity of stain desired. In this short time the lignified tissues became deeply stained, while the parenchyma and meristem remained lighter in color. In the case of guayule, the violet also allows contrast with the red-staining Sudan IV, if this stain for rubber is used. The exact timing of each step was not critical, and as the material remained in each solution for only a few moments, the whole process required less than half an hour. Two people working together were able to keep both the cutting and the staining-mounting processes in continuous operation, making ten to twelve finished slides per hour.

For regular areas, such as pith, xylem, and phloem, linear measurements were found most suitable. The image of the section to be measured was projected against a screen which was marked into quarters by two lines crossing at right angles. It was then possible to center the projected image and measure it systematically. Four diameter measurements were made of the pith of the stem. The zones of tissue, such as xylem and bark of the stem, were measured along four radii, one in each quarter of the section. Some selection was necessary in making these measurements because of the irregular pattern of vascular bundles or fiber tissue. The average of the four measurements was used to calculate the

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area of any one tissue. This was done by means of standard formulas for the area of a circle or of the zone between two circles. The ruler used was marked according to the magnification of the image, so that linear measurements

TABLE 1

VARIATION IN TISSUE AREAS OF THREE CONSECUTIVE SECTIONS FROM EACH OF FOUR BRANCHES OF GUAYULE. FIGURES REPRESENT AREAS IN SQUARE MILLIMETERS CALCULATED ON BASIS OF AVERAGE OF FOUR LINEAR MEASUREMENTS

| No. of section | Branch no. | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | Pith | | | |
| 1 | 0.69 | 0.55 | 0.85 | 0.69 |
| 2 | 0.69 | 0.58 | 0.85 | 0.69 |
| 3 | 0.72 | 0.53 | 0.85 | 0.64 |
| | Xylem | | | |
| 1 | 9.71 | 6.24 | 5.49 | 6.85 |
| 2 | 9.83 | 5.93 | 5.85 | 6.47 |
| 3 | 10.15 | 5.90 | 5.58 | 6.53 |
| | Bark | | | |
| 1 | 17.49 | 12.53 | 11.91 | 14.51 |
| 2 | 17.57 | 12.65 | 12.32 | 14.24 |
| 3 | 18.55 | 12.90 | 12.13 | 14.24 |

were read directly in millimeters and areas were calculated in square millimeters. Reproducibility of the results is illustrated by measurements of tissue areas in three consecutive sections taken from a comparable internode of each of several branches (table 1). In the case of guayule, the symmetrical arrangement of tissues lent itself readily to this type of analysis.

A totally different problem is encountered in measuring numerous irregular areas, such as bast fibers. A simplified area photometer, manufactured by the American Instrument Company and now commercially available, was used in such cases. The instrument operates on the principle of interception of a light beam focused on a photoelectric cell and measurement of the resulting difference in energy output of the cell in terms of scale divisions.[3]

In order to measure fiber areas, the images were first projected on to a screen over which a circular piece of clear cellulose acetate, 13 inches in diameter, had been fastened. An outline of each fiber group in a representative quarter of a stem was then traced on the acetate sheet, and later these traced areas were filled in with ink. By placing the acetate bearing the inked tracings in the photometer, a reading indicating the amount of light intercepted was quickly obtained, and this reading was later converted into square millimeters of tissue by means of a calibration curve.

In inking the tracings, Roberts' India ink, to which 25% glycerin had been added in amounts of 1 cc. to 10 cc. of ink, was found satisfactory. This mixture was readily washed from the sheets so that they could be used repeatedly. As a precautionary measure, the sheets were tested for constancy and uniformity of light absorption at intervals during their use.

The method for calibrating the instrument was important. To obtain comparable measurements of the known areas used for calibration and the unknown areas of fiber tissue, it was necessary that the instrument be calibrated

[3] MITCHELL, J. W., Measurement of the area of attached and detached leaves. Science 83:334–336. 1936.

with the known areas inked on cellulose acetate, in the same manner as that used in the case of unknown areas. Known areas of suitable sizes were therefore inked on the acetate and read in terms of scale divisions. For convenience, the sizes of these known areas were adjusted to the degree of magnification, so that it was possible to plot a curve with which readings in photometer scale divisions were converted directly into square millimeters of stem tissue. In order to test the reproducibility of results obtained with the photometer, the known areas of different sizes were measured repeatedly, and variation between the replicated readings was found to be relatively small (table 2).

TABLE 2

VARIATION IN REPLICATED PHOTOMETER READINGS OF KNOWN AREAS. READINGS EXPRESSED IN TERMS OF SCALE DIVISIONS

| KNOWN MAGNIFIED AREA (CM.²) | EQUIVALENT TISSUE AREA (MM.²) | READINGS | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 11.04..... | 0.5 | 11.8 | 12.5 | 11.8 | 11.9 |
| 22.08..... | 1.0 | 13.4 | 13.8 | 13.5 | 13.6 |
| 33.12..... | 1.5 | 15.3 | 15.3 | 15.2 | 15.2 |
| 44.16..... | 2.0 | 16.7 | 16.9 | 16.8 | 17.2 |
| 55.20..... | 2.5 | 18.2 | 18.3 | 18.4 | 18.5 |
| 66.24..... | 3.0 | 20.0 | 20.0 | 20.2 | 20.2 |

APPLICATION OF METHOD.—Data from a preliminary study of stem tissues of guayule, *Parthenium argentatum*, illustrate an application of the procedures described. Seedlings of four strains were grown under comparable conditions outdoors. Six months after planting, ten plants from each group were selected for tissue measurements. A cross section was made through the true stem at a level approximately ½ inch above the lowest leaf scars. Area measurements of the tissues were recorded and the data analyzed statistically.

In the case of guayule, it is desirable to have an accurate knowledge of the extent of the bark, those tissues between the vascular and cork cambiums, since this is the major rubber-containing region of the plant. By means of linear measurements, the width and percentage of bark in the stems were determined and the data summarized (table 3). These data indicate that variation in average width and percentage of bark in the cross sections was relatively small, but the percentage of bark in strain B was significantly more than that in plants of strain C, by odds of 19:1. Measurements of the pith, a second rubber-containing tissue, showed that the average percentage area occupied by pith in strain D was 6.9, an amount significantly less than that of strains A and C, which measured 10.9 and 11.1, respectively.

TABLE 3

AVERAGE AMOUNT OF BARK IN CROSS SECTIONS OF STEMS AND PERCENTAGE FIBER IN BARK OF FOUR STRAINS OF GUAYULE PLANTS (TEN PLANTS OF EACH STRAIN)

| STRAIN | BARK | | PERCENTAGE FIBER IN BARK | |
|---|---|---|---|---|
| | Width (mm.) | Percentage of entire section | Minimum | Maximum |
| A..... | 1.39 | 58.3 | 18.7 | 33.8 |
| B..... | 1.31 | 60.2 | 10.5 | 30.8 |
| C..... | 1.31 | 56.1 | 24.8 | 37.2 |
| D..... | 1.29 | 57.8 | 16.3 | 40.2 |

Measurements of the amount of fiber in the bark of guayule are used to illustrate the application of the method in determining areas of tissues irregular in outline. Quantitative results show that seedling plants varied to a great

extent in amount of fiber, and in some of the plants this nonrubber-producing tissue occupied a relatively large proportion of the total cross-sectional area (table 3, fig. 1).

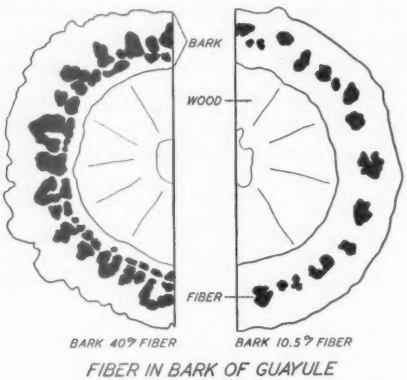


FIG. 1.—Variation in amount of fiber in bark of two plants of two strains of guayule. Plants grown under comparable conditions and sections cut from comparable positions on stem. Diagrams represent tracings of projected images. Fiber areas darkened in a manner similar to that used in making measurements of irregular areas.

In many crop plants, substances of economic importance are produced in special structures, such as rubber in the latex system of *Hevea;* or in specific regions or tissues, such as quinine in the bark of cinchona or fibers in the stems of hemp and flax. Through the application of the method described it is possible to measure anatomical variations which could not be readily determined on the basis of the usual visual method of examination. Furthermore, it permits evaluation of anatomical structures on a quantitative basis useful to the plant breeder in selecting individuals with the most desirable structures and constituents and to the physiologist in determining degrees of structural response to different cultural treatments.

## Summary

1. A method of measuring areas of tissues in numbers of plants is described. Sections of the plant were cut with the aid of a freezing microtome, stained, and mounted in glycerin jelly. By means of a projection microscope, an image of the section was thrown on a screen, and areas of tissue symmetrical in outline were calculated from linear measurements, while irregular areas were traced on cellulose acetate, inked, and measured by means of an area photometer.

2. This method is of value in accurately determining the areas or volumes of specific tissues associated with specific functions, such as rubber storage in guayule.

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# WINTER HARDINESS IN JUVENILE AND ADULT FORMS OF CERTAIN CONIFERS[1]

V. R. GARDNER

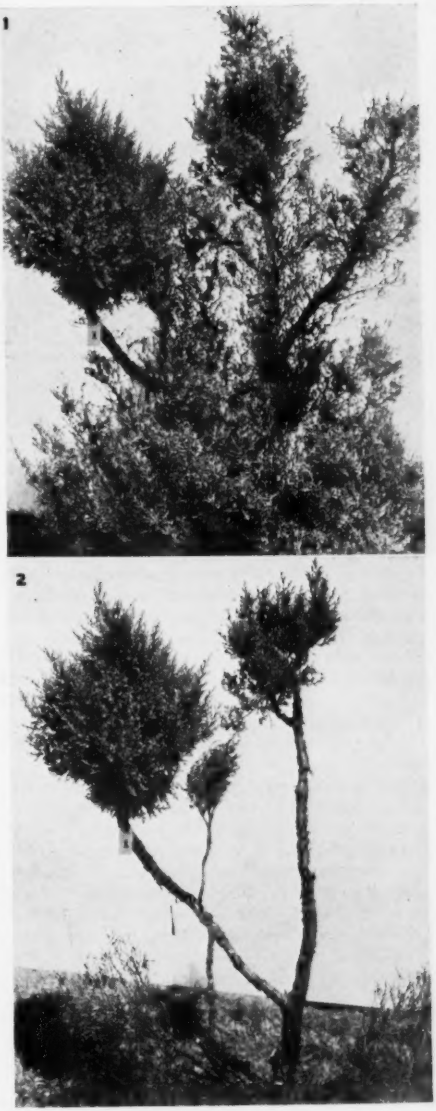
Anatomical and morphological differences between juvenile and adult forms of many conifers have long been noted. Numbers of juvenile and intermediate, as well as of adult, forms have been named, propagated, and introduced into cultivation because of the distinctive characters of their foliage. For the most part these involve shape and color differences of leaves only,

[1] Journal Article no. 669 (n.s.), Michigan Agricultural Experiment Station.

although often associated with such foliage differences are striking variations in general habit of growth. Few observations have been recorded on substantive or physiological differences among these several forms of the same species or on the same plant, although it would seem that—especially in those instances where reversion occurs in individual branches of otherwise normal plants—ideal material would be available for such studies.

Winter injury to a number of plants of *Juniperus chinensis* var. *variegata* and *J. horizontalis* growing on the grounds of the Graham Horticultural Experiment Station near Grand Rapids, Michigan, afforded an opportunity to record some rather striking differences in respect to hardiness. The plants had been set in 1925, had received good care, and had grown well. Those of *J. chinensis* were 6–7 feet tall and had a spread of 4–6 feet in the fall of 1942. Those of *J. horizontalis* were 18–24 inches in height and had a spread of 6–8 feet. Both varieties are characterized by adult appressed, scale-like leaves, but both occasionally produce reverted branches having typical acicular, juvenile-type foliage. These particular plants had produced a number of such reverted branches that were 3–5 years old at the end of the 1942 growing season. The 1942 shoot growth was considerably greater and more vigorous on the juvenile than on the adult portions of the plants and therefore theoretically should have been less mature in the fall.

The winter of 1942–43 was not especially severe, the minimum temperature probably not going below −12° F. Nevertheless many conifers considered winter-hardy in this territory suffered severe killing-back of twigs and branches; in some cases entire plants were destroyed.




FIGS. 1, 2.—Fig. 1, old plant of *J. chinensis* var. *variegata* showing three branches whose terminal portions have reverted to juvenile condition. Entire lower portion and large branch at right have normal adult-type foliage. Fig. 2, plant shown in fig. 1, with winter-killed adult foliage and branches trimmed away.

FIG. 3.—Terminal of old branch (enlarged close-up view of righthand branch shown in fig. 2). Left fork has reverted completely to juvenile condition; right fork shows normal adult-type foliage of the variety, except for several small reverted twigs.

In the spring of 1943 practically all the adult leaves, and the small twigs bearing them, on these two forms of *Juniperus* were dead, while the leaves and wood of the reverted juvenile branches were uninjured. Apparently most of the adult foliage and twigs that survived had had the advantage of some protection by snow. In no instance had the branches bearing juvenile foliage had any advantage of location, either from the standpoint of exposure to minimum temperature or from wind or sunlight. It was clearly a case of a difference in hardiness of tissues between the two forms (figs. 1–3).

In the case of *J. horizontalis*, reverted branches bearing juvenile foliage had struck root more freely where they came in contact with the soil than had branches of the normal form with adult foliage.

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# CURRENT LITERATURE

*Fundamentals of Cytology.* By LESTER W. SHARP. New York and London: McGraw-Hill Book Co., 1943. Pp. x+270. Illustrated. $3.00.

This volume is intended solely as a textbook, and therefore is more than a mere revision of the same writer's well-known *Introduction to Cytology.* Since it is not intended as a work of reference, the author has been free to select for discussion the topics appropriate to an introductory course. Eleven chapters deal with cell structures and functions; five with problems of cytogenetics; and the final chapter with cytology and taxonomy. In harmony with the general plan, the discussion of each topic is brief and so necessarily follows the author's judgment as to what is most important and presents his conclusions in debatable fields. Upon the judgment as to the author's success in these two respects of those teachers who use it, or who consider using it, will obviously depend the ultimate usefulness of the work. The impression of the reviewer is that Professor SHARP has been remarkably successful in both his selection and his discussion. His writing is clear and forceful; recent results are adequately considered, although possibly with a certain tendency to minimize those which are older but still sound; and the conclusions are in general judicious and conservative. A cytologist examining the book may note the absence of some topics he would have included and disagree here and there with the author's views.

All references to literature are omitted in the text, in harmony with the general plan of simplification. The extensive bibliography of the previous work is replaced by a short list for each chapter of discussions—chiefly of a general nature. Terminology has been somewhat simplified. Possibilities here are necessarily limited; so many conceptions are involved in the consideration of scientific results which can be expressed only in technical terms that the demand voiced by many laymen—and an occasional teacher—for a discussion of the results of research in every-day language is a demand for the impossible. Where usage is still inconsistent, a choice of terms must be made; and the author's choice will not in every case be that of the critical reader. It may be asked, for example, why "monoploid" rather than "haploid"; why "kinetochore" instead of "centromere"?

The illustrations, many of them new, are excellently prepared and effectively reproduced.

It is to be hoped that before too long there may be a revision of the author's previous book which, freed now from the limitations of space necessitated by its previous double function, will continue the tradition of an inclusive reference work.—C. E. ALLEN.

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*Plant Viruses and Virus Diseases.* By F. C. BAWDEN. 2d ed. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. xi+294. Illustrated. $4.75.

The type of the first edition was lost with the invasion of the Netherlands. For this second edition BAWDEN has rewritten more than half the chapters and made various modifications in the others. The most extensive alterations have appeared in discussions of inactivation, of the properties *in vitro,* and of the relationships to insect vectors. Attention is given chiefly to the viruses and relatively little to the virus diseases as such.

The various topics receiving major consideration are similar to those in the first edition, namely: introductory survey; symptomatology; transmission; relationships between viruses and their insect vectors; strains, mutation, and acquired immunity; serological reactions; properties, including optical characteristics; inactivation; sizes of virus particles; physiology of virus-diseased plants; classification of viruses; control of virus diseases; and origin and multiplication of viruses.

Some of the controversies active when the first edition appeared have been resolved and so receive less space. For example, there is now little if any question that the specific protein isolated from an infected plant represents the virus itself. It is accepted that the tobacco mosaic virus is a nucleoprotein instead of a globulin. Concerning controversies still active, the reader will agree or not with BAWDEN, depending on his own view.

The discussion continues as to whether or not a virus is a living entity, but a somewhat different emphasis has been indicated. The present difficulty seems to lie less in our ignorance of viruses than in deciding what criteria to apply to determine whether a substance is alive or not.

The merits of different classifications are still actively debated. Those based upon symptoms have usefulness in certain directions. However, BAWDEN thinks that any lasting system, like those of pathogenic bacteria and fungi, should be based on the intrinsic properties of the viruses themselves and not on those of the host. As all the viruses isolated so far are nucleo-proteins, it is suggested that the methods of classifying proteins may be helpful in making major groups of viruses. Considerable emphasis has been given to the value of serological reactions and other properties.

In relation to mutations, BAWDEN has indicated that some of the evidence is inadequate. The assumption that local lesion technique always gives pure viruses seems to provide a false feeling of security.

Most of the work on plant viruses has been done by pathologists, although in recent years co-operating chemists, crystallographers, entomologists, geneticists, serologists, physicists, and others have been attracted by this intriguing field. While much is known about the viruses themselves, the author finds that little is known about their behavior within the host plant, and this aspect may provide the next major advance. In this direction some valuable methods are already available, which BAWDEN has not discussed. They include, for example, well-known studies of respiratory enzymes and the production of virus-like symptoms (ZIMMERMAN, P. W.,

Contrib. Boyce Thompson Inst. 12:1–14. 1941) with naphthoxyacetic acid and many other known chemicals.

This second edition is a useful and welcome compilation. Since the research on plant viruses has contributed so much to an understanding of their fundamental nature, this book is valuable to the animal as well as to the plant biologist. It is a fine tribute to British appreciation of fundamental science that this excellent work has been continued while bombs were falling.—A. J. RIKER.

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*An Introduction to Pollen Analysis.* By G. ERDTMAN. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. xvi+239. Illustrated. $5.00.

Since its virtual beginning in 1915, the literature of pollen analysis has grown until it now approaches 1700 titles, in many languages. Workers are widely scattered, largely self-taught. From 1927 until 1940, Dr. ERDTMAN has published lists of the literature and maintained close contacts with workers.

The present book presents in a practical and direct way the essentials of technique and procedure. The historical section is brief, and little attempt is made to summarize results, except as they serve to elucidate method. A rather detailed chapter on the chemistry of peat is included for this reason.

The heart of the book consists of six chapters on pollen identification. These, too, are essentially practical in character, rather than "morphological" in the wider sense. Use is made of the methods of description to which WODEHOUSE has made such substantial contribution. The chapter on analysis of tertiary deposits should be of general interest to botanists, as this phase of study is likely to yield increasingly valuable results.

American readers should keep in mind the aim of the book as just set forth. While it is unlikely that they will employ the elaborate symbolism for records and diagrams which has been developed in Europe, it is important that they understand it as presented here. On the other hand, a genetic discussion of peat and related sediments would have been most welcome on this side of the Atlantic, where information of the kind to be found in Vol. 1, *Handbuch der Moorkunde*, is not readily available. BARKLEY's contribution to the statistical principles of counting probably deserves more than a mere citation.

The difficulties under which this book has been produced would justify far greater limitations than are to be found in it. It is not often that a book with a botanical title can offer so much to workers in so many fields of science. Archaeology, climatology, ecology, floristics, forestry, geology, and limnology will all benefit by those improvements in the method and application of pollen analysis which are the chief concern of this excellent volume.—P. B. SEARS.

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*The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms; A Colour Atlas and Guide.* By T. WALLACE. London: His Majesty's Stationery Office, 1943. Pp. vi+116. Pls. 114. 10s.

This book was written primarily for the use of technical officers and advisors concerned with problems of crop production, for progressive farmers, and for vegetable and fruit growers. It contains elementary discussions of plant nutrition, the ability of soils to supply mineral nutrients to the plant, and methods of determining mineral deficiencies in crops. Of more interest is the discussion of visual symptoms of various mineral deficiencies in horticultural and agricultural crops commonly grown in England. A key is included for the recognition of the most important symptoms in various crops for diagnostic purposes.

The main contribution of the book is the collection of colored photographs, many of which are excellent, although others leave much to be desired. In general, only the gross external appearance of the plant is shown; photographs illustrating individual deficiency symptoms in detail would have been desirable. Nevertheless, the collection in this easily available form, representing the results of the author's experience and study in the field of mineral nutrition, is an important contribution.—C. B. LYON.